The validity and reliability of intraocular pressure measurement using rebound tonometry in children with ocular and systemic disease

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Summary

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Objective: This study examined three main objectives: 1. The validity of Rebound Tonometry (RBT) measurements in children. 2. The reliability of suboptimal RBT readings and the relationship between co-existing characteristics and these measurements. 3. The reliability of suboptimal RBT measurements in children with heritable connective tissue disease (HCTD).
Design: A cross-sectional study design was used for objectives 1 and 2 and a case control study was used for objective 3.
Setting: The Eye Department of Birmingham Women's and Children's Hospital (BWCH).
Participants: Fifty children were recruited, including 34 with glaucoma for objectives 1 and 2 and 16 for objective 3 (8 HCTDs, 8 healthy controls).
Interventions: RBT measurements were taken at the geometric centre of the cornea of one eye (RBT_{on}) and at 3 mm temporally (RBT_{off}), followed by Goldmann tonometry (GAT). Additional data regarding sex, age, nystagmus, strabismus, type of glaucoma, treatment, visual acuity, spectacle prescription, ethnicity, health and corneal scars were recorded from the participants' clinical notes. The same procedure was conducted on 8 children with HCTD and 8 controls
Results: Mean RBT_{on} was significantly higher than GAT by 2.4 (SD 3.0) mmHg. A statistical difference was found between the age groups and the IOP status ($p < 0.05$). Mean RBT_{off} readings were not significantly different from RBT_{on} in children with glaucoma ($p = 0.100$) and this difference was not associated with co-existing characteristics ($p > 0.05$). Mean (RBT_{off} - RBT_{on}) was not significantly different between children with HCTDs and healthy controls ($p = 0.06$).
Conclusion: This study achieved its main objectives and found that:

- RBT_{on} measurements differ from GAT but are useful clinically.
- The relationship between RBT_{on} and GAT varies with the age of the child.
- Suboptimal RBT_{off} measurements are reliable in children with glaucoma with a range of co-existing conditions and in children with HCTDs.

Key words: glaucoma, intraocular pressure, cornea, anterior chamber, heritable connective tissue disease.

Dedication

This Doctorate is dedicated to my husband Abbas and my children Tom, Alice and Max, who encouraged me as I travelled frequently up and down to Birmingham to conduct this study.
It is also dedicated to the children of Birmingham Women's and Children's Hospital.

Acknowledgements

This study was a result of collaboration between Aston University and Birmingham Women's and Children's Hospital (BWCH). Special thanks to Professor Leon Davies, Dr Nicola Logan and Dr Richard Armstrong of Aston University and all the staff of the Eye Department at BWCH including consultant ophthalmologist Mr Joseph Abbott and orthoptists Laura Ramm, Bavnesh Sond and Monica Chauhan, without whose help this study could not have been conducted. I also want to thank the children and parents of the Eye Department of BWCH for their incredible enthusiasm and positivity when taking part in this study.

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All photographs taken by the author and Prof Leon Davies

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"A dripping tap fills a bucket" (Laura Ramm, 2018).

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Abbreviations

Abbreviation	Description
AL	Axial length
ARS	Axenfeld-Rieger Syndrome
ASD	Anterior Segment Dysgenesis
BCH	Birmingham Children's Hospital
BWCH	Birmingham Women and Children's Hospital
BIG	British Infantile and Childhood Eye Study
BMI	Body Mass Index
CC	Corneal curvature
CCT	Central corneal thickness
CH	Corneal hysteresis
CI	Confidence Interval
CMGs	Clinical management guidelines (College of Optometrists)
COMET	Correction of Myopia Evaluation Trial
CRF	Corneal resistance factor
CRF	Case report form
DALK	Deep Anterior Lamellar Keratoplasty
DCT	Dynamic Contour Tonometry
ECH	Extra cellular matrix
EDS	Ehlers Danlos Syndrome
EGPS	European Glaucoma Prevention Study
EMGT	Early Manifest Glaucoma Trial
GAT	Goldmann applanation tonometer
HCTD	Heritable Connective Tissue Disease
HRA	Health Research Authority
ICC	Intra Class Coefficient
IOP	Intraocular pressure
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomized Control Trial Number
JOAG	Juvenile Open Angle Glaucoma
KP	Keratoplasty
MFS	Marfan's Syndrome
MYOC	Myocilin gene
NICE	National Institute for Clinical Excellence
NTG	Normal tension glaucoma
OCR	Ocular response analyser
OHT	Ocular Hypertension

OHTS	Ocular Hypertension and Treatment Study
OI	Osteogenesis Imperfecta
PCG	Primary Congenital Glaucoma
PIS	Patient Information Sheet
POAG	Primary Open Angle Glaucoma
PK	Penetrating keratoplasty
RBT	Rebound tonometer
RBT_{off}	Rebound tonometry, 3 mm temporal to the geometrical centre of the cornea
RBT_{on}	Rebound tonometry, at the geometrical centre of the cornea
REC	Research Ethics Committee
RF	Rheumatoid Factor
Rx	Refractive error
SEL	Simple Ectopia Lentis
TP	Tono-Pen
UKEGS	UK & Eire Glaucoma Society
UKPGS	UK Paediatric Glaucoma Society
UKISCRS	UK & Ireland Society of Cataract &Refractive Surgeons
WGA	World Glaucoma Association

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Chapter 1

Literature review

1.1 Introduction

Childhood glaucoma is a rare but sight threatening disease that occurs in babies and children. Raised intraocular pressure (IOP) can cause progressive optic nerve atrophy, which if untreated can lead to irreversible loss of vision and blindness (Tham *et al*., 2014). IOP reduction is the single most important modifiable factor in the treatment of the disease (Weinreb *et al*., 2014). Therefore, it is important to be able to measure IOP accurately and reliably. Goldmann applanation tonometry (GAT) is considered to be the gold standard clinical method of measuring IOP (Munkwitz *et al*., 2008). However, it involves the use of topical anaesthetic eye drops and the applanation of the cornea by a cone. As this is not well tolerated by young children and babies, general anaesthetics have been used in the past in order to be able to measure IOPs. The advent of the iCare rebound tonometer (RBT) in 1997 has changed this (Grigorian *et al*.,2015). RBT tonometry uses a small probe that briefly touches the cornea. It causes minimal discomfort and is tolerated by young children and babies without the need for eye drops and has therefore, reduced the need for general anaesthetics. RBT has revolutionised IOP measurement in paediatric eye departments (Dahlmann-Noor *et al*., 2013). However, there is a paucity of data concerning the validity and reliability of RBT measurement in children (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013). Studies in adults have indicated that RBT measurements may overestimate IOP when compared with GAT (Fernandes *et al*., 2005; Davies et al., 2006; Muttuvelu et al., 2012). RBT readings should ideally be taken from the centre of the cornea (Beasely *et al*.,2013). However, children often look away during measurement due to the upward movement of the eyes as a result of Bell's phenomenon which occurs with attempted lid closure, leading to suboptimal RBT measurements (Mustafa, 2005). As far as the author is aware little is known about the validity of suboptimal RBT measurements. Children with glaucoma often have co-existing conditions e.g. strabismus, nystagmus and corneal scars that can make IOP measurement more challenging (Flemmons *et al*., 2011b). As far as the author is aware little is known about the association of these co-existing conditions and suboptimal RBT measurements. Further, as far as the author is aware nothing is known about the validity of

suboptimal RBT measurements in children with heritable connective tissue disorders (HCTD) such as Marfan's and Ehlers Danlos syndromes, whose corneas may be affected by their conditions (Sultan *et al*., 2002; Pesudovs, 2004).

Therefore, the present study had three main objectives and examined the following:

1. The validity of RBT measurements in children with glaucoma (Experiment 1).
2. The reliability of suboptimal RBT readings in children with glaucoma and the association of their co-existing conditions (Experiments 2 & 3).
3. The reliability of suboptimal RBT measurements in children with HCTDs (Experiment 4).

For objectives 1 and 2 a prospective observational study design was used to compare RBT with GAT and suboptimal RBT with central corneal measurements in children with glaucoma. Thirty-four children with glaucoma were recruited for these objectives. A control group was not included in the design because the majority of the children attending the Eye Department of BWCH had some sort of eye disease and only children who were having their eye pressures measured as part of their routine care could be included. Therefore, it was not practical to recruit controls for these objectives within a reasonable time frame.

For objective 3 a case control study design was used to examine the reliability of suboptimal RBT measurements in children with HCTD's. Therefore, an age matched control group was also recruited. However, this was feasible within the time frame allowed as only 8 were needed.

In this chapter background information regarding the structure, embryology and development of the anterior eye is given. An overview of Instrumentation used to measure IOP is also presented, in order to demonstrate the usefulness of rebound tonometry. In addition, IOP, children's glaucoma and HCTDs will be described. Knowledge of these subjects is necessary in order to understand the relationship between the anterior eye and IOP measurement in children with glaucoma and HCTDs.

1.2 Structure of the Eye

The eye is an enclosed globe which sits in the orbital cavity of the skull and can be divided into two segments. The anterior chamber between the cornea and iris and the posterior chamber between the iris, ciliary body and lens are found in the anterior segment. The vitreous chamber, retina, choroid and sclera are found in the posterior segment (Snell and Lemp,2013) (Fig.1).

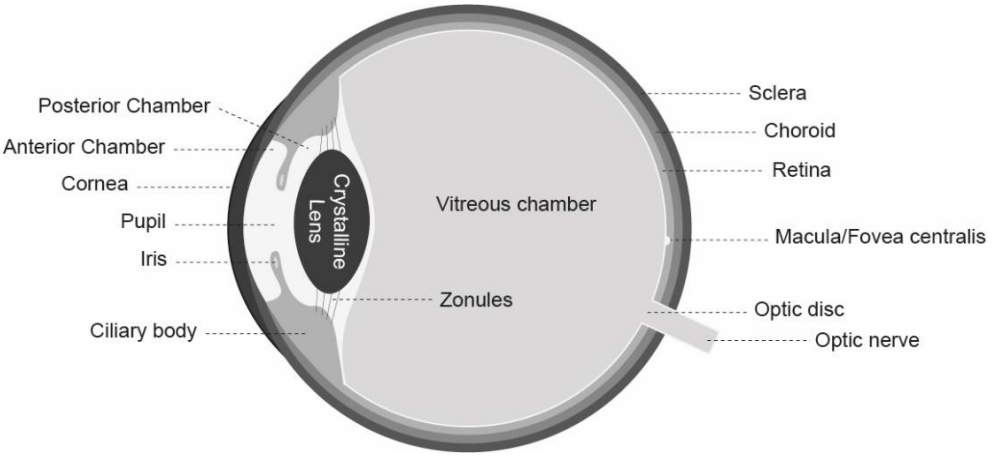


Fig. 1. *The Structure of the eye. The anterior eye consists of the anterior chamber and posterior chambers with the cornea Iris pupil, lens with associated zonules and ciliary body. The posterior eye consists of the vitreous chamber, retina, choroid and sclera. The diagram shows the position of the macula optic nerve and disc: Adapted from Snell and Lemp, 2013.*

1.2.1 Anterior chamber

The anterior chamber contains aqueous fluid and is situated between the endothelium of the cornea anteriorly and the trabecular meshwork, part of the ciliary body and the root of the iris peripherally. The posterior border is formed by the anterior surface of the iris and the pupillary zone of the lens. It is deepest at the centre and narrows towards the periphery (Remington, 2012) (Fig 2).

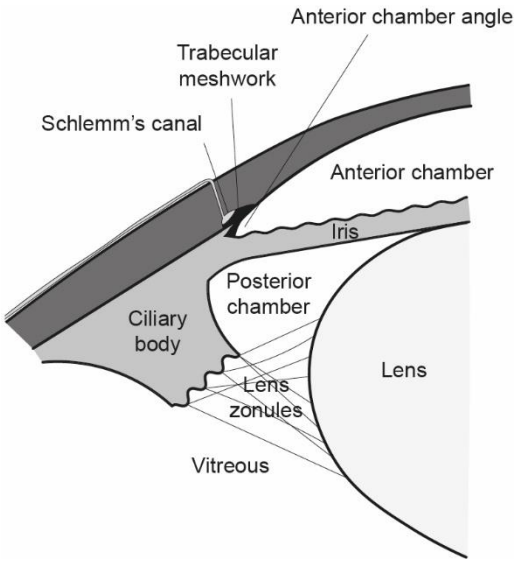


Fig. 2. *Cross section of the anterior segment of an adult eye indicating the position of the structures. The angle of the anterior chamber is a complex area where the anterior part of the scleral wall meets the cornea. The edge of the sclera has three areas i.e. the corneoscleral junction, the scleral spectrum and the spur, which is short and marks the border of the trabecular meshwork (Snell and Lemp, 2013, Sampaolesi, 2009). The scleral spectrum and the spur create a posterior channel in which Schlemm's canal and the trabecular meshwork can be found (Moses and Grodzki,1977). The majority of aqueous exits the eye from this filtration channel (Sampaolesi, 2009). Adapted from Snell and Lemp, 2013.*

1.2.2 Aqueous Fluid

Aqueous fluid generates and maintains IOP (Tamm *et al.*, 2015). It also brings nutrients to the cornea and lens and aids the exit of waste products (Remington, 2012). Aqueous is formed at a rate of 2 µl/min by hydrostatic passive leakage of fluid from the blood vessels and the active transport of sodium and other ions by non-pigmented epithelial cells which are situated on the ciliary body in the posterior chamber. The aqueous fluid circulates in the eye exiting in the anterior chamber mainly through the trabecular meshwork into Schlemm's canal, with a smaller proportion exiting via the uveal scleral outflow mechanism (Kniestedt *et al.*, 2008). Raised IOP affects trabecular outflow whereas uveal scleral outflow is fairly constant and is not affected (Remington, 2012).

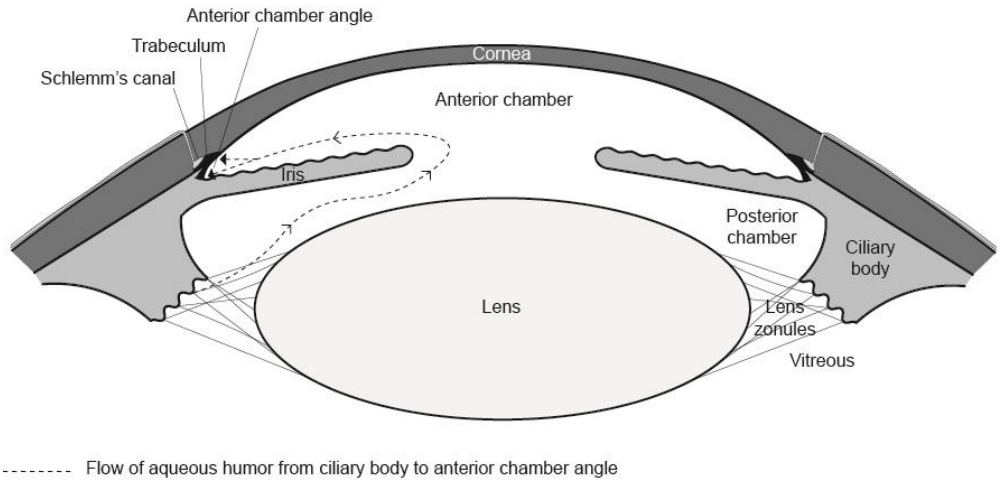


Fig. 3. *The anterior chamber of the eye with the dotted line and arrows showing the flow of the aqueous from the non-pigmented ciliary epithelium of the posterior ciliary body into the anterior chamber. The aqueous fluid exits via the trabecular meshwork and uveal scleral pathway. Adapted from Snell and Lemp, 2013.*

1.3 Embryology of the eye and anterior segment development

Following implantation of the embryo in the womb, ectoderm and endoderm layers evolve from the inner cell mass of the trophoblast. At 15 days the primitive streak arises from the ectoderm and forms the intra embryonic mesoderm leading to a tri-laminar embryonic disc with the eye deriving from the ectoderm and mesoderm (Sampaolesi, 2009). The neural tube develops from neural ectoderm and gives rise to the optic vesicle at week 4, which then folds back on itself to form a cup-shape at 5 weeks (Riordan-Eva, 2011). A crystalline lens vesicle then develops, which separates from the surface ectoderm at 6 weeks when corneal differentiation occurs and by the 7th week the lens nucleus has formed (Hoar, 1982). The iris, ciliary body and ciliary processes begin to develop and by the 3rd month the anterior chamber is formed and mesenchymal tissue can be seen in the anterior chamber angle (Sampaolesi, 2009). Table 1 demonstrates the embryonic origin of the structures of the eye. In the 4th month Schlemm's canal and the trabecular meshwork develop with the 5th month bringing further development of Schlemm's canal due to vasculature changes at the corneal/scleral transition area (Hamanaka *et al.*,1992) Mesodermal reabsorption starts, the core of the trabeculae evolve, the aqueous humour begins to form, there is further mesodermal reabsorption and the ciliary muscle starts to be displaced posteriorly (Sampaoloesi, 2009). Aqueous humour drainage begins around this time and increases with the development of the foetal eye (McMenamin, 1989). Barkan (1938) suggested that a pre-trabecular endothelial membrane

covers the anterior chamber and that this fenestrates in the 7th month. He postulated that lack of fenestration can restrict aqueous outflow. However, electron microscopy has shown that this membrane does not exist (de Luise, 1983). In months 8 and 9, the posterior displacement of the ciliary muscle speeds up and completes the angle recess and the reabsorption of the mesoderm continues. By the age of 5 years the recession of the chamber angle is complete (Sampaoloesi, 2009). A failure in the correct development of the anterior chamber can lead to congenital glaucoma (Fig.4) and will be discussed further in section 1.5.

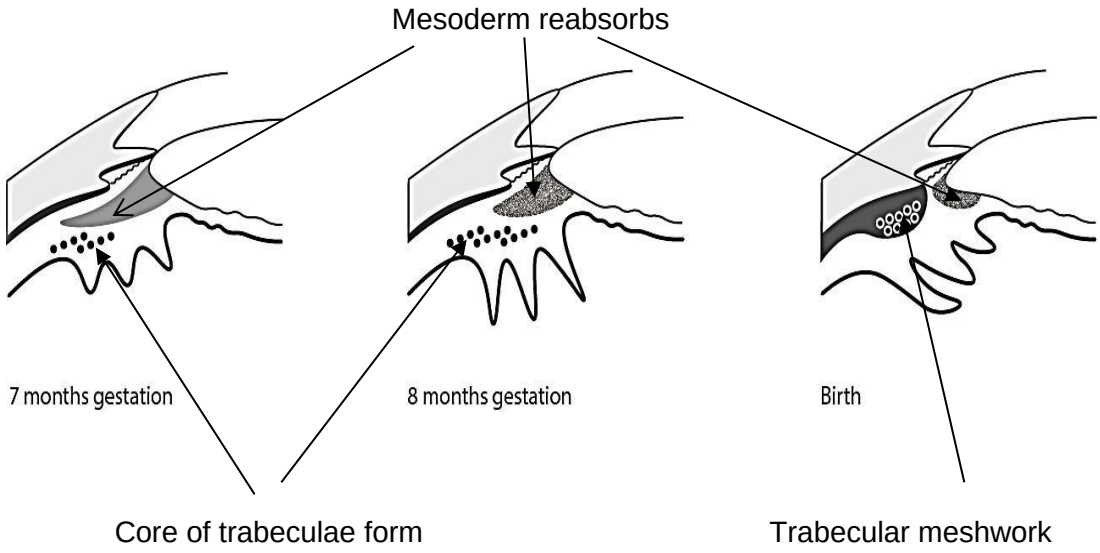


Fig. 4. *Formation of the anterior chamber in a normal eye from gestation month 7 until birth. In congenital glaucoma the anterior chamber fails to develop fully and can resemble months 7-8. The schematic diagram shows the development of the trabecular meshwork and the reabsorption of the mesoderm, remnants of which can be present at birth. Adapted from Sampaoloesi (2009).*

Embryonic origin	Structure
Surface ectoderm	-Lacrimal gland -Epithelium of the cornea -Conjunctiva -Adnexal glands -Epidermis of the eyelids
Neural crest (arises from surface ectoderm)	-Corneal keratocytes -Endothelium of the cornea and trabecular meshwork -Stroma of the iris and choroid -Ciliary muscle -Fibroblasts of the sclera, vitreous and Optic nerve meninges
Neural Ectoderm	-Optic vesicle -Optic cup -Retina -Retinal pigment epithelium -Pigmented and non-pigmented Layers of the ciliary epithelium -Posterior epithelium -Dilator and sphincter muscles of the iris -Optic nerve fibres and glia -Vitreous
Mesoderm	-Extraocular and eyelid muscles -Orbital and ocular vascular endothelium

Table 1. *Embryonic origin of the ocular structures. Adapted from Riordan-Eva, (2011). The anterior segment of the eye develops from the mesenchyme of the neural crest. Failure of neural crest migration leads to abnormalities of the anterior chamber which can result in early onset glaucoma (Tian et al., 2012).*

1.4 Intraocular pressure

IOP is the internal pressure of the fluid contained within the eye (NICE, 2009). It is measured in the Standard International (SI) metric unit mmHg which is also used in medicine as a

measurement of blood pressure (Bergeson and Smith,1981). Mean IOP in a White adult population is approximately 16 mmHg (Eysteinsson *et al*., 2002). Stamper *et al*. (2009b) also found that the normal IOP range in adults is between 7 and 21 mmHg with the upper limit representing +2 standard deviations from the mean 16 mmHg, which represents 95% of the population. IOP is pulsatile and fluctuates due to the rhythmic variation of blood supply to the eye (Langham *et al*., 1989).

Children have lower IOPs than adults and as they grow their IOPs rise (Fig.5). At six months old a normal healthy IOP for an infant is 8 mmHg, rising by 1 mmHg each year. By the age of 5 years the average normal IOP is 12-14 mmHg and by 12 to 15 years this rises to 15-17mmHg (Sampaolesi, 2009). As far as the author is aware Flemmons *et al*., (2011b) is the only previous study that has examined the association of the age of children (with glaucoma), with the difference between RBT and GAT. They did not find an association between age and RBT readings that were above GAT. As there is such a paucity of data, the present study was designed to address this.

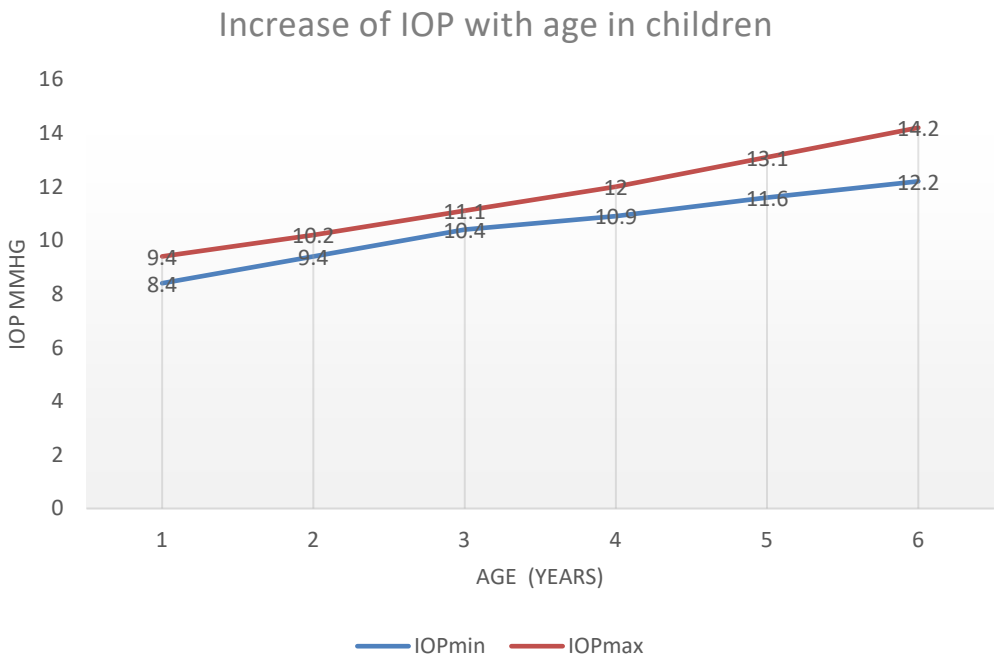


Fig. 5. *Shows maximum (IOP_{max}) and minimum (IOP_{min}) IOP as a function of age. It is interesting to note the increase of IOP with age. Adapted from Sampaoloesi (2009). No standard deviations were available.*

IOP in normal healthy eyes can fluctuate during the day from between 3 to 4 mmHg, with the peak mostly occuring between 6 to 8am. Therefore, single IOP measurement may not reflect

the true level (Phelps *et al*.,1974). Short term factors that can affect IOP include food and fluid intake, exertion and changes in systemic blood pressure, whereas diurnal variation may be the result of endogenous factors such as cortisol production (Wilensky,1991).

Twenty four hour monitoring is useful to detect peaks in IOP. Diurnal variation can be larger in patients with glaucoma; a study by Tajunisah *et al*., (2007) compared 202 adult eyes with known or suspect glaucoma with 100 healthy controls and found that IOP varied by 6 mmHg in the known or suspect glaucoma group and by 4 mmhg in the healthy controls. As far as the author is aware, there is a paucity of information in the literature concerning diurnal variation in children with glaucoma. A small study of 10 patients aged 19 to 38 years with JOAG, Merritt *et al*., (1979) found that peaks of IOP ocurred both at 6pm and at midnight. Hsiaro *et al*., (2012) used the rebound tonometer to examine diurnal variaition in IOP in 22 eyes of 11 healthy children and found that IOP varied by 4 to 6 mmHg, was higher earlier in the day and lower at the end. Likewise Flemmons *et al*., (2011b) in their rebound tonometry study of 17 eyes of children with known and suspect glaucoma found that 45% of IOP peaked in the morning and 43.5% expeienced a trough in the evening. Further study is needed on diurnal variation of IOP in both healthy children and in those with glaucoma.

1.5 Glaucoma

Glaucoma is a major cause of irreversible blindness worldwide in adults, with a global prevalence of 3.54% in those aged 40 to 80 years. Worldwide in 2013 it was estimated that 64.3 million adults had glaucoma and this is projected to increase to 111.8 million in 2040 (Tham *et al*., 2014). Elevated IOP can lead to glaucoma, which occurs when aqueous production is too high or the outflow is restricted. A common reason for raised IOP is increased trabecular resistance outflow (Kniestedt *et al*., 2008). Outflow resistance can occur in the juxtacalicular connective tissue and inner wall endothelium of Schlemm's canal due to stiffening of the cells in this region and may well be a critical causative factor of primary open angle glaucoma (POAG), which is the most common type of glaucoma found in adults (Tamm *et al*., 2014). However, the causes of dysfunctional trabecular outflow differ between adults and children making childhood glaucoma a different disease (Sampaolesi, 2009). In children, outflow resistance tends to occur due to developmental abnormalities, congenital defects,

acquired ocular disease and systemic disorders (Papadopoulos *et al*., 2007) and will be discussed in more detail in the following section.

1.5.1 Paediatric glaucoma

Paediatric glaucoma also known as childhood glaucoma has been defined by the World Glaucoma Association (WGA) as IOP related damage to the eye (Beck *et al*., 2013). This differs from adult POAG which has been described as a progressive optic neuropathy (Weinreb and Kaur, 2004). Raised IOP, changes to the optic disc and visual field loss are found in both childhood and adult glaucoma. However, other structures (see 1.5.4) are also affected in children due to their anatomical and physiological differences (Biglan, 2006). In addition to IOP measurement and optic disc examination, diagnosis in children is also based on corneal diameter and axial length (Borrego Sanz *et al*., 2016).

Although it is relatively rare in children primary congenital glaucoma (PCG), is the leading cause of congenital blindness and is responsible for 5% of world blindness in children (Papadopoulos *et al*., 2007). An ophthalmologist in the Western world in a non-specialist centre can expect to see a new case only once every five years (Walton, 1979). The British Infantile and Childhood (BIG) Eye Study (2007) found that in the United Kingdom the annual incidence of PCG was 1:18,000 live births. In the Republic of Ireland, it was 1:30,200. They also found that children of Pakistani origin had an incidence nine times higher than White populations (Papadopoulos *et al*., 2007). In the Indian state of Andra Pradesh the prevalence is as high as 1:3,300 births (Dandona *et al*., 2001). It is more common in cultures where consanguinity occurs due to intermarriage of relatives (Gencik, 1989). PCG is more common in males than females with a ratio of 2:1 (McGinnity et al., 1987) and is bilateral in 80% of cases (François, 1980).

Treatment of paediatric glaucoma varies according to the type of glaucoma and the age of the child at onset. Medical management is often the treatment of choice for secondary glaucoma and is used as an adjunct to surgery for congenital and infantile cases (Papadopoulos and Khaw, 2007). Surgical treatment to aid filtration includes trabeculectomy and pressure sensitive shunts (Biglan, 2006).

CRGN classification of childhood glaucoma
Primary:
PCG
JOAG
Secondary, associated with:
Non-acquired ocular abnormalities
Non-acquired syndrome or systemic disease
Acquired systemic disease
Following cataract surgery

Table 2. *Classification of childhood glaucoma by the CRGN. PCG (Primary Congenital Glaucoma) occurs as a result of malformation of the anterior segment (anterior segment dysgenesis). JOAG (Juvenile Open Angle Glaucoma) is an aggressive open angle glaucoma that occurs under the age of 40. Both have strong genetic links. Adapted from: Hoguet et al. (2016). Glaucoma after cataract surgery is the most common secondary glaucoma (Brookes, 2012), others include glaucoma due to JIA (Juvenile Idiopathic Arthritis), (Sabri et al., 2008) and following anterior segment trauma (Agrawal et al., 2013).*

In the past childhood glaucoma has been classified in different ways, from age of onset, inheritance, associated systemic diseases, to anterior segment anomalies (Yeung *et al*., 2010). More recently an international body, the Childhood Glaucoma Research Network (CGRN) introduced a new classification system based on clinical findings, the timing of onset and the context in which glaucoma is found. The CGRN categorizes childhood glaucoma as primary or secondary (Table 2.)

1.5.2 Primary glaucoma and non-acquired ocular abnormalities and syndromes

Anterior segment dysgenesis (ASD) covers a wide spectrum of disorders that affect the iris, cornea, trabecular meshwork and Schlemm's canal, 50% of which will have glaucoma (Idrees *et al*., 2006). Classification can be been made difficult by the complex nature and overlap in clinical presentation; however, there is a strong genetic link (Ito and Walter, 2014).

PCG is an ASD that affects the filtration system of the anterior chamber, resulting in an increase in IOP (Idrees *et al*., 2006). New born PCG is noted at birth and infantile PCG occurs in the first year of life. It is the most severe type of childhood glaucoma and requires immediate surgery. However, it can be recognised late (Sampaolesi, 2009). Children with PCG are born with isolated trabeculodysgenesis which was originally thought to be caused by

Barkan's membrane as previously mentioned in section 1.3. However, it is now thought that excess collagen within the trabecular meshwork is the most likely cause (Brookes, 2012). The majority of PCG is non-hereditary and sporadic, but 10 to 20% of cases follow an autosomal recessive hereditary pattern (François, 1980).

Juvenile-onset primary open angle glaucoma (JOAG) occurs in older children and is an autosomal dominant disease with high penetrance which like adult POAG has been associated with mutation of the myocilin gene (MYOC) (Bruttini *et al*., 2003). Age of onset is not always known but has been defined as 5 to 40 years (*Willoughby et al*., 2004). However, JOAG is different from the most common adult form of glaucoma POAG as it occurs at a younger age, tends to be more aggressive and can lead to severe visual impairment (Wiggs *et al*., 1996). Unlike POAG, JOAG is a rare disease. In 2016 the CGRN examined a group of 204 children with glaucoma and found that only 7.4% had JOAG (Hoguet *et al*., 2016).

Sturge-Weber syndrome (SWS) is an encephalotrigeminal angiomatosis that can lead to glaucoma, raised episcleral venous pressure and congenital angle abnormality. It can affect the central nervous system, the skin and the eye and has an incidence (rate of occurrence per year) of 1:50000 live births with glaucoma occurring in 50% of cases (Brookes, 2012).

The ASD Axenfeld -Rieger Syndrome (ARS) is a rare disorder with a prevalence (number of cases at one time) of 1/ 50 000 to 1/100 000 in infants (Seifi and Walter, 2018). Ocular manifestations of ARS include iris hypoplasia, correctopia, polycoria, posterior embryotoxon and lens subluxation. These patients may also have systemic defects of the face, teeth, heart and abdomen (Seifi and Walter, 2018). ARS has an autosomal-dominant inheritance and is caused by embryonic malformation of the anterior chamber associated with mutations of *FOXC1, FOXC2* and *PITX2* transcription genes (Smith *et al*., 2000). *PITX2* and *FOXC1* activate in the first three months of pregnancy and precise levels are required for the anterior chamber to develop normally (Brookes, 2012). Low levels can result in poor development of the trabecular meshwork and Schlemm's canal leading to ASD and associated bilateral glaucoma (Brookes, 2012). Some inductive transcription factors emanate from the lens and mutations can lead to congenital cataracts, iris coloboma and opaque corneas (Cvekl and Tamm, 2004).

Peter's anomaly is another example of an ASD caused by abnormal *PITX2* and *FOXC1* genes (Brookes, 2012). The corneal endothelium, Descemet's membrane, and posterior stroma may be affected which can result in corneal opacification (Idrees *et al*., 2005). Iris hypoplasia, iridocorneal adhesions, corectopia and glaucoma may also be present (Cvekl and Tamm, 2004). It is a rare condition noticed at birth, is usually associated with other systemic complications, occurs in both sexes and can be unilateral or bilateral (Bhandari *et al*., 2011). Corneal transplantation is often necessary to enable the development of vision in these children (Dana *et al*., 1997).

Congenital microphthalmia is a rare disorder with a prevalence of 30/100,000 and is responsible for 11% of blindness in children (Verma and FitzPatrick, 2007). The eye is small with a reduced volume and there may also be an associated coloboma, orbital cysts and other systemic abnormalities (Tucker *et al*., 1996). Primary genetic defects are associated with microphthalmia. However, congenital rubella, toxoplasma and the herpes virus may also be responsible (Ragge *et al*., 2007). Children with glaucoma may present with a range of co-existing conditions i.e. nystagmus, strabismus and corneal scars, which can make IOP measurement more difficult (Flemmons at al., 2011b).

1.5.3 Childhood glaucoma secondary to systemic disease and cataract

Childhood glaucoma can occur as a result of other diseases that interfere with the drainage of aqueous fluid by the filtration angle (Sampaolesi, 2009). Aphakic glaucoma following cataract surgery is the most common secondary glaucoma found in children and can develop several years later (Chak and Rahi, 2008). The British Congenital Cataract Study (BCCS) recorded all children with newly diagnosed congenital/infantile cataract over the period of one year from 1995 to 1996 (Rahi and Dezateaux, 2001). Out of this cohort,165 children underwent cataract surgery (275 eyes). Six years later, Chak and Rahi (2008) noted that that the annual incidence of pseudophakic glaucoma in this cohort was 5.25 per 100 eyes per year, with glaucoma occurring from 0.39 months to 6.73 (median = 1.34) years after surgery. Early detection of congenital cataract is important in the prevention of amblyopia. However, their study found that early age of cataract diagnosis was the single most important factor associated with the development of glaucoma post-surgery, with a 10x increase of age at

detection associated with 64% reduction in hazard ratio (95% CI 41%-79%; $p < 0.001$). Other risk factors include type of cataract surgery, postsurgical uveitis and primary intraocular lens implantation (Magnusson et al., 2000). Microphthalmos is also a risk factor for aphakic glaucoma (Biglan, 2006).

A longitudinal study conducted over 18 years in Sweden found a 12% incidence of aphakic glaucoma in children with congenital cataract (Magnusson *et al*., 2000). Johnson and Keech (1996) found a prevalence of 32% over a seven-year period following cataract surgery at a young age.

The mechanism behind the development of pseudophakic glaucoma is unclear. It may a reaction to post-operative inflammation in very young eyes or anterior chamber abnormalities associated with congenital cataract. In addition, cataract surgery in the very young can itself result in a high number of complications (Chen et al., 2006).

Uveitic glaucoma is associated with Juvenile Idiopathic Arthritis (JIA). One third of patients with JIA develop secondary ocular complications and most of those with uveitis tend to be female (Sabri *et al*., 2008). One fifth of patients with JIA who are negative to rheumatoid factor (RF) may develop a chronic nongranulomatous anterior uveitis which is often bilateral and some children with the HLA B27 gene can also develop anterior uveitis (Kanski, 1990). It can be hard to treat and severe vision loss has been reported in 18 to 28 % of patients with JIA associated uveitis (Thorne *et al*., 2007).

Glaucoma diagnosis, needs two of the following:
Disc cupping: increasing, asymmetry of cup disc ratio > 0.2, rim thinning Cornea: Haab striae, large diameter; > 11 mm new born, > 13 mm any age Myopia: progressive with abnormal growth Field defect: glaucomatous
Glaucoma suspect, needs one of the following:
IOP: > 21 mmHg measured at two separate visits Disc cupping: suspicious Field defect: suspicious Corneal diameter/axial length: abnormally large

Table 3. *Diagnosis of glaucoma by the CRGN. Adapted from: Hoguet et al. (2016).*

1.5.4 Clinical findings

Children's glaucoma is similar to adult types in that it is associated with raised IOP and progressive optic atrophy (Biglan, 2006). However, raised IOP in babies and young children can result in bupthalmous where the horizontal corneal diameter increases, the eye looks enlarged and Haab striae develop due to cracks in Descemet's membrane giving rise to corneal haze (Morales *et al*., 2013). Optic nerve cupping due to the elasticity of the tissue in this area can develop, but with good IOP control this can reverse (Biglan, 2006). Myopia due to increased axial length occurs in two thirds of children with PCG, astigmatism > 2 D occurs in a quarter, amblyopia is common and infants may present with epiphora, photophobia and blepharospasm (Biglan., 2006). Older children with JOAG do not exhibit external signs and are examined in a similar way to adults with POAG with IOP and visual field measurements (Beck, 2001). In 2016 the CGRN published a summary of clinical findings that can be used to aid the diagnosis of childhood glaucoma (Table 3).

1.6 The cornea

The cornea together with the sclera forms an outer tunic of connective tissue that protects the internal structures of the eye and resists the IOP thereby maintaining its shape (Remington, 2012). It is transparent allowing light to enter the eye and focus on the retina (Davson, 1984). The limbus is a transitional zone and forms a border between the cornea and sclera (Van Buskirk, 1989). The cornea along with the anterior chamber and lens forms part of the optical system of the eye and the majority of the refractive power is attributed to its anterior surface which is ellipsoidal in shape (Kiely *et al*., 1982). The Reykjavik Eye Study examined the corneal curvature, central corneal thickness and IOP in 925 healthy White subjects and found that the adult cornea in males had an average anterior radius of curvature (CC) of 7.8 mm (SD 0.60). In females a slightly steeper average CC of 7.6 mm (SD 0.58) was found (Eysteinsson *et al*., 2002). Adult corneas have an average central thickness of 0.53 mm and peripheral thickness of 0.71 mm (Remington, 2012). Children's central and paracentral corneal thickness increases with age (Hussein *et al*., 2004). Various studies, using different methods (see Fig. 6), have estimated the age at which CCT reaches adult levels. Hussein *et al*. (2004) estimated that this occurs between the ages of five to nine years. Ehlers *et al.,*

(1976) and Muir *et al*., (2004) state that this occurs about the age of 3 and the Paediatric Eye Disease Investigator Group (PEDIG) (2011) found that CCT increases every year from the age of one reaching a plateau of 573 µm by the age of eleven (see Fig. 6). Knowledge of the development of CCT in children is important because CCT has been found to influence IOP measurement (Hansen and Ehlers, 1971), which will be discussed further in section 1.6.3.

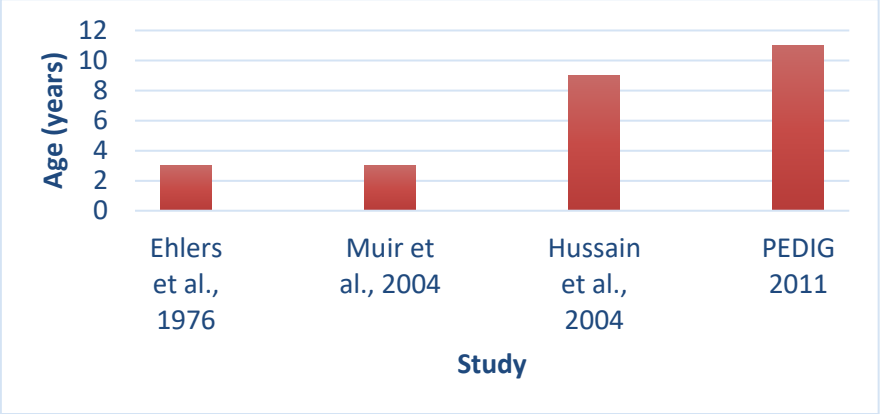


Fig. 6. *Studies have estimated the age at which children's corneal thickness reaches adult levels. However, these studies used different instrumentation to measure CCT, which may explain the different results. Ehlers et al., (1976) used an optical method, Muir et al., (2004) used an unnamed ultrasound pachymeter, Hussein et al., (2004) used the DGH-2000 ultrasound pachymeter (DGH Technology, Inc., Frazer, Pennsylvania) and PEDIG used the handheld Pachmate (DGH Technology, Inc., Exton, Pennsylvania.)*

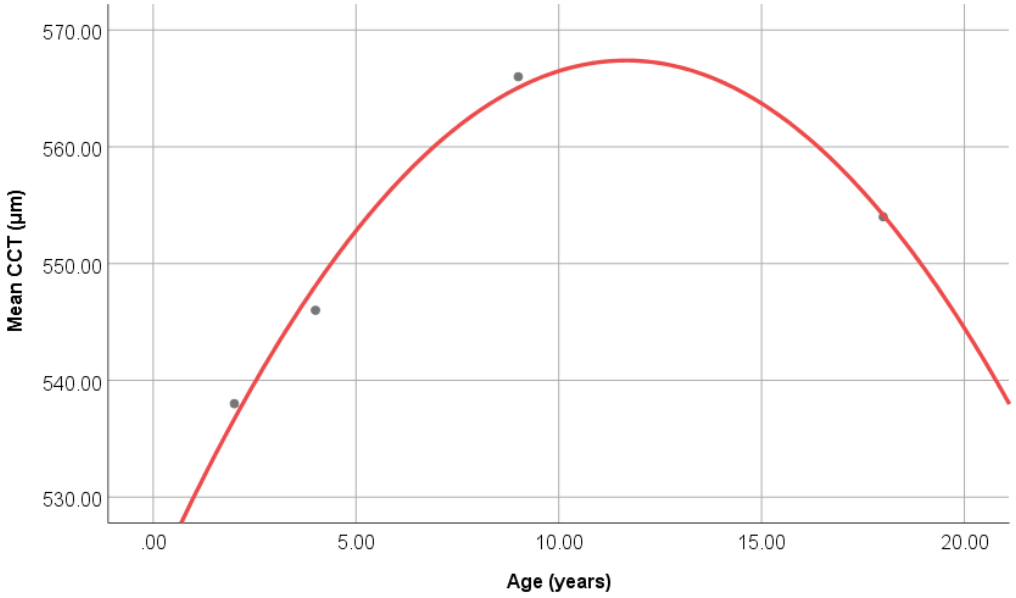


Fig. 7. *Graph adapted from a study by Hussein et al. (2004). They found that between 6 to 23 months mean CCT was 538 (SD 40) µm and from 2 to 4 years mean CCT increased to 546 (SD 41) µm. From 5 to 9 years CCT increased to 566 (SD 48) µm and between 10 to 18 years mean CCT reduced slightly to 554 (SD 35) µm.*

It is interesting to note that Hussein *et al*. (2004) found that average CCT in children increased with age reaching adult levels somewhere between 5 to 9 years and reduced slightly thereafter (Fig. 7).

1.6.1 Structure of the cornea

Traditionally the cornea has been described as a structure with 5 layers with the outermost consisting of non-keratinized stratified epithelium that sits on a basement membrane underneath which Bowman's layer is found. Below this lies the stroma beneath which Descemet's membrane and the endothelium are situated (Meek and Knupp, 2015). Recently, a new pre-Descemet's posterior stromal layer (Dua's layer) has been proposed and will be discussed in more detail further on.

The epithelium at the centre of the cornea consists of 5 to 7 layers of cells and is approximately 53 μm thick (Reinstein *et al*., 2008). Peripherally the epithelium is thicker with layers of 7 to 10 cells (Shridhar, 2018). Outer layers of the epithelium are flattened with protective tight junctions and have microvilli which help with retention of the tear film (Sridhar, 2018). The middle layer consists of wing shaped cells which have both tight and gap junctions and below this is a single layer of columnar cells that are anchored to the basement membrane by type VII collagen fibrils (Remington, 2012). The transparency of the corneal epithelium is a result of the homogeneity of the refractive index of its cells (Dohlman, 1971).

Bowman's layer is found below the basement membrane of the corneal epithelium, is 8 to 14 μm thick and consists mainly of strong randomly interwoven fibrils of collagen types I, III, V and VII (Wilson and Hong, 2000, Lagali *et al*., 2009).

The stroma accounts for 90% of corneal thickness and is composed of glycoproteins (collagen) that sit within an extracellular matrix (ECM) consisting of water, proteoglycans (keratocan, lumican and decorin), soluble proteins, inorganic salts, keratocytes, lymphocytes, macrophages and polymorphonuclear leukocytes (Jalbert and Stapleton, 2005). Keratocytes are thin specialized fibroblasts that produce and maintain collagen fibrils and the extracellular matrix (Wilson *et al*., 2001). There are approximately 200 lamellae in the stroma, within which bundles of predominately type I collagen fibrils are found (Davson, 1984). The lamellae are axially staggered and run parallel to the surface of the cornea (Meek and Boote, 2004). They

are approximately 31 nm in diameter which increases to 34nm with age (Meek and Leonard, 1993; Meek and Boote, 2004). Fibrils within each individual lamella run parallel with each other; however, fibrils of adjacent lamellae have different orientations and this can affect corneal curvature (Newton and Meek, 1998). Fibrils found in the stroma are similar to collagen found elsewhere in the body; however, the axial periodicity of collagen fibrils in the stroma is 65 nm compared with 67 nm in tendons (Meek and Boote, 2004). Collagen fibrils have a slight crimp in them, with the distribution of crimp angles changing with mechanical load. This gives rise to the pliability and mechanical response of the cornea preventing deformation of the optically important central region (Liu *et al*., 2014). Lamellae are more closely packed together at the centre of the cornea than at the periphery; therefore, although the centre of the cornea is thinner, it has greater resistance (Boote *et al*., 2003). (Fig. 8). Anterior lamellae are more highly interwoven than elsewhere and insert into Bowman's membrane with a "bow spring-like" design with angles averaging 19 degrees, which helps to maintain the shape of the anterior cornea and provides biomechanical support (Morishige *et al*., 2006). Lamellae in the middle of the stroma tend to be transverse in nature and posterior lamellae are orthogonal and more easily damaged (Winkler *et al*., 2011). Peripheral lamellae outside the central 4 mm area become interwoven at deeper levels than the centre and join the circumcorneal annulus at the limbus. In addition, anchoring lamellae enter the stroma in alignment with the extra ocular muscles (Meek and Knupp, 2015). Although the lamellae arrangement helps to maintain the overall structure of the cornea, it is the arrangement of the fibrils within the lamellae and the lack of pigment and blood vessels in the stroma that contributes to corneal transparency (Meek and Knupp, 2015). This arises from the interference of light scattered by the collagen fibrils due their precise organization within the ECM (Sridhar, 2018).

More recently a plane of cleavage has been found in the most posterior part of the stroma during deep anterior lamellar keratoplasty (DALK) procedures, which gives rise to a 10-20 μm thick pre-Descemet's layer of posterior stroma named Dua's layer (Dua *et al*., 2013). It consists of several layers of type 1 collagen lamellae, has few keratocytes and continues into the trabecular meshwork (Dua and Said, 2016).

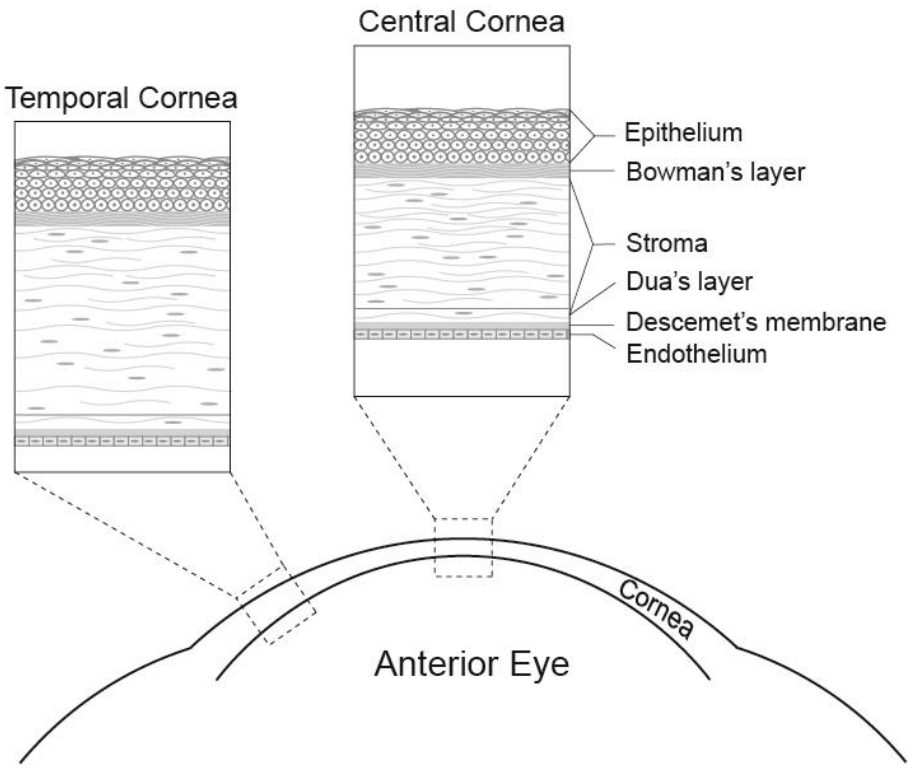


Fig. 8. *Schematic diagram of the central and peripheral corneal structures. Differences in thicknesses are due to the more closely packed lamellae in the centre. Adapted from: Dua et al. (2013). The present study examined the reliability of temporal corneal RBT measurements as this is a good alternative position to the centre when taking RBT measurements in uncooperative children.*

Descemet's membrane is found just below the stroma and is approximately 7–10 μm thick and is the basement membrane of the endothelium, mainly consisting of type IV collagen and laminin (Sridhar, 2018). Below this is the corneal endothelium, which is a single layer of flattened squamous epithelial polygonal cells with tight junctions their main function is to maintain corneal hydration (Remington, 2012).

Corneal pathology affecting any one or more of the corneal layers can result in an increase of light scatter and reduction in corneal transparency (Meek and Knupp, 2015).

1.6.2 Embryology and development of the cornea

Corneal differentiation in the human embryo begins at around the 33rd day of development when the lens vesicle separates from the neighbouring surface ectoderm and mesenchymal cells from the neural crest migrate anteriorly (Sampaolesi, 2009). The first migration forms the corneal endothelium, the second forms the stroma and by the 5th week both the corneal epithelium and endothelium can be seen (Riordan-Eva and Cunningham, 2011). Bowman's

layer is first seen between 13 and 19 weeks and is formed from the anterior stroma (Sevel and Issacs, 1988). Evidence suggests that the corneal epithelium may also play a role in its formation, with type V collagen filaments extending from the basement membrane of the epithelium into the anterior stroma at 13 weeks gestation (Tisdale *et al*., 1988). Bowman's layer may be absent in congenital diseases such as Peter's anomaly, type II osteogenesis imperfecta and Ehlers-Danlos Syndrome where it can break down leading to stromal haze (Wilson and Hong, 2000). Decemet's membrane begins formation at 8 weeks inutero by secretion from endothelial cells with a banded layer secreted before birth and an unbanded after (Sridhar, 2018).

Mutations of the genes that encode collagen and fibrillin can result in weakened connective tissue structure within the cornea (as mentioned above) and other structures of the eye and will be discussed in more detail further on in this chapter.

1.6.3 Biomechanical properties of the cornea

CCT is a well-known property of the cornea that can affect IOP measurement, with thicker corneas recording higher IOPs (Hansen and Ehlers, 1971). In the mid1990s, the Ocular Hypertension Treatment Study (OHTS) examined 1637 participants with a mean age of 55 years and a mean IOP of 24.9 (SD 2.7) mmHg (Gordon and Kass, 1999). It was a multicentre randomized clinical trial which looked at the benefit of the use of ocular hypotensive medication in preventing the onset of primary open angle glaucoma (POAG) in patients with ocular hypertension (OHT). One its major findings, was that CCT is a strong predictor for the development of POAG within 5 years, with thinner corneas most at risk (Gordon *et al*., 2002). The European Glaucoma Prevention Study (EGPS) confirmed these findings (EGPS, 2007). The OHTS also found a difference in CCT between ethnicities, with African-American participants having thinner corneas than Whites. Above average thickness of above 600 μm was found in 25% of participants and may have resulted in underestimation of true IOP (Brandt *et al*., 2001). Ehlers *et al*. (1975) measured cannulated IOPs in patients undergoing cataract surgery and found that GAT and cannulated IOP are closest when CCT is 520 μm. Using Ehler's correction of 7 mmHg/100 μm deviation from a CCT of 520 μm (for IOPs measured by GAT) the OHTS found that 50% of participants actually had higher IOPs than

first thought (Ehlers *et al*., 1975; Brandt, 2007). As a result, patients can be misclassified. Those with a normal tension glaucoma (NTG) diagnosis may in fact have POAG and those with raised IOP and no other signs may simply be healthy eyes with thick corneas (Brandt, 2007). The association of CCT and the optic nerve head has been the subject of several studies. The Early Manifest Glaucoma Trial (EMGT) of participants with OHT and POAG found that the lamina cribrosa moved further forwards in those thin CCT than in those with thick CCT when IOP was lowered (Leske *et al*., 2003). CCT has also been associated with disc size with thicker corneas tending to have smaller discs and thinner corneas having larger (Pakravan *et al*., 2007). Evidence suggests that CCT may be inherited; CCT in Greenland Eskimos was found to have a heritability correlation of between 0.6 and 0.7 (Alsbirk, 1978). The Twin Eye Study of identical and non-identical twins found a CCT heritability correlation of 0.95 in the identical twin cohort which suggests that CCT is highly heritable (Toh *et al*., 2005). Thin corneas have been found in heritable diseases such as congenital glaucoma, osteogenesis imperfecta (OI), Marfan Syndrome (MFS) and Ehlers-Danlos Syndrome (EDS) (Everekliogl u *et al*., 2002; Sultan *et al*., 2002; Henriques *et al*., 2004; Pesudovs, 2004). More about these heritable conditions will be discussed further on in this chapter. Their association with IOP measurement using RBT and GAT, will be discussed later on in chapters 3 and 5. Viscoelastic properties also influence the elasticity and resistance of the cornea and the influence of CCT on IOP readings can vary according to corneal rigidity (Medeiros and Weinreb, 2006). Liu and Roberts (2005) used a mathematical model to show that variations in corneal elasticity in the normal population can produce an IOP measurement error of up 17 mmHg which is higher than that produced by CCT. They also demonstrated that elasticity governs the influence of CCT on IOP measurements with stiff corneas having a steep relationship between IOP and CCT. Corneal hysteresis (CH) is a measurement that reflects the elasticity of the cornea and can be measured by the Ocular Response Analyser (ORA) which will be described further in section 1.8.5.

CH varies in normal eyes and has been found to be reduced in eyes with corneal diseases such as keratoconus and Fuchs dystrophy due to the disorganized pattern of the collagen lamellae found in the stroma (Kirwan *et al*., 2006). Patients with POAG and NTG have low hysteresis due to biomechanical changes which may in turn be related to pathology at the

lamina cribrosa (Kirwan *et al*., 2006). LASIK and conductive keratoplasty also affects CH due to changes in corneal structure. Unlike IOP, CH does not exhibit significant diurnal variation and is independent of IOP (Chui *et al*., 2008; Luce, 2005).

Kirwan *et al*. (2006) found that healthy children's corneas have a similar CH to adults with a mean of 12.5 (SD 1.35) mm Hg and does not vary with age. Children with congenital glaucoma have a lower CH of 6.3 (SD 1.58) mm Hg with a range of 3.1 to 8.5 mm Hg (those with Haab striae and large corneal diameters having the lowest). CH characterises the corneal resistance factor (CRF) which represents the overall resistance of the cornea due to corneal thickness, rigidity and curvature hydration etc. and is also measured by the OCR (Medeiros and Weinreb, 2006). Perucho-González *et al*. (2017) demonstrated that CH and CRF can be used to distinguish between patients with primary congenital glaucoma and healthy controls due to structural and biomechanical differences. Both CH and CRF can affect RBT readings (Chui *et al*., 2008). It has also been noted that CH is strongly associated with risk of development and progression of glaucoma in adults ((Luz *et al*., 2016). CH also tends to be abnormal in connective tissue diseases like Marfan's syndrome (Kara *et al*., 2012). There is a paucity of information about the validity and reliability of RBT in children with glaucoma and connective tissue disease, so this study is designed to address this.

1.7 Connective tissue disease

Connective tissue provides the scaffolding for the body's tissues and consists of cells and an ECM. It is found in organs, blood vessels, nerves, the lymphatic system and muscles and is the site of the management of many complex systems (Pavelka and Roth, 2015). Connective tissue disease is a broad term used to describe a range of conditions. Some arise from acquired autoimmune inflammatory conditions where the body attacks itself by making autoantibodies. This includes Systemic lupus erythematosus (SLE), Dermatomyositis, CREST syndrome, Scleroderma, Rheumatoid arthritis, Reiter's syndrome, Behçet's syndrome, Poly arteritis nodosa, and Panniculitis (Weller *et al*., 2015). Other forms are inherited due to genetically defective connective tissue (Grahame, 2000). As connective tissue defects can affect the structure of the eye, the present study examines the use of RBT in children with inherited diseases, rather than acquired.

1.7.1 Heritable disorders of connective tissue (HCTD)

HCTDs are genetically inherited connective tissue disorders that affect collagens and fibrillins which are proteins that form the connective tissue matrix which is found all over the body in the eyes, blood vessels, joints and skeleton (Grahame, 2000). Mutations in the genes for collagen types I, II, III, IX, X and XI lead to diseases of the bone, cartilage and blood vessels (Kuivaniemi *et al*., 1997). Mutations in fibrillins lead to Marfan's Syndrome (MFS) and congenital contractural arachnodactyly (CCA) (Olivieri *et al*., 2010)

The four principle HCTDs are:

1. MFS
2. Ehlers-Danlos syndrome (EDS)
3. Osteogenesis imperfecta (OI)
4. Benign joint hypermobility syndrome (BJHS).

Joint laxity and hypermobility are found in all four conditions (Grahame, 2000).

Stickler syndrome is an autosomal connective tissue disorder that also affects the eye (Snead and Yates, 1999). The ocular effects of these diseases are listed in Table 4.

HCTDs are a heterogeneous group of disorders making them difficult to differentiate between and classify (Beighton *et al*., 1988). In 1986 experts met in Berlin to decide how to classify them. The resulting "Berlin Nosology" listed the diagnostic manifestations, genetics and requirements necessary for the diagnosis of the main HCTDs including MFS and EDS. It was noted that the basis of defects was not always known (Beighton *et al*., 1988). Several years later the genetic cause of MFS was identified leading to changes to the Berlin Nosology. The new "Ghent Nosology" ruled out MFS in patients with a family history of the disease who had non-specific findings, which was an important refinement as a diagnosis of MFS can be stigmatizing and affect career choice. Further refinement of the Ghent Nosology in 2010 led to more accurate classification of MFS, differentiating it from other HCTDs such as familial ectopia lentis (Loeys *et al*., 2010).

The Berlin Nosology also did not differentiate between the different types of EDS adequately. Therefore, in 1997 the revised "Villefranche Nosology" was published. The new nosology classified EDS into six subtypes according to the clinical phenotype which correlated to the underlying collagen defect (Beighton *et al*., 1998, Jobling *et al*., 2014). More variants of EDS

have since been identified leading to a refinement of the Villefranche Nosology (De Paepe and Malfait, 2012) and this will be discussed in more detail later on in this chapter.

The Beighton scoring system is used in the diagnosis of HCTD. A goniometer measures joint hyperextension, with both sides of the body being evaluated. The score is added together (Table 5).

HCTD	Ocular manifestation
Marfan's syndrome	- Lens subluxation - Retinal lattice degeneration - Axial myopia - Angle anomaly/glaucoma - Flattened cornea - Blue sclera
Ehlers-Danlos syndrome	- Keratoconus - Epicanthal folds - High myopia - Strabismus - Amblyopia - Blue Sclera - Microcornea - Lens dislocation - Brittle cornea - Retinal detachment
Osteogenesis imperfecta	- Blue sclera - Low ocular rigidity - Microcornea - Optic neuropathy
Benign joint hypermobility syndrome	- Blue sclera - Droopy eyelids - High myopia - lens dislocation - Antimongoloid slant
Stickler syndrome	- High myopia - Lattice degeneration - Retinal detachment - Cataract/ lens dislocation - Ptosis - Strabismus

Table 4. *The ocular effects of HDCTs. Adapted from: Beighton (1970); Snead and Yates (1999); Maumenee (1981); Evereklioglu, C et al. (2002); Fikree et al. (2013).*

Condition	Points score
Hyperextension of little finger >90^0	1 + 1 = 2
Thumb touches forearm	1 + 1 = 2
Elbow extends >10^0 with arm straight	1 + 1 = 2
Knee bows backwards >10^0 when standing	1 + 1 = 2
Can place palms of hands flat on the ground with straight legs	1 = 1

Table 5. *The Beighton scoring system for hyperextensibility with a maximum score of 9 points from the two sides of the body: adapted from Malfait et al. (2017).*

Marfan syndrome

Dr Antoine Marfan first noticed the skeletal characteristics of what later became known as the Marfan syndrome in 1896. In 1931 autosomal dominant inheritance was discovered and in 1943 the serious cardiovascular complications were first described (Cross and Jenson, 1973). Groth *et al*. (2014) found a prevalence of 6.5/100,000 in a Danish population with no difference between men and women and a median age at diagnosis of 19 years. MFS results from mutation of the fibrillin-1encoding gene (FBN-1), leading to fibrillin abnormalities (Kara *et al*., 2011). Fibrillin is a glycoprotein that provides the scaffolding for elastic microfibrils. It is an important component of the ciliary zonule and is arranged in parallel bundles in the zonular fibres providing strength and elastic recoil (Ashworth *et al*., 2000). Connective tissues of the corneal stroma, corneal epithelial basement membrane, the conjunctiva, the iris, ciliary body and processes and the endothelium of Schlemm's canal all contain fibrillin. It is also found in the posterior segment of the eye in Bruch's membrane, the choroid, the scleral stroma and the lamina cribrosa (Wheatley *et al*., 1995). Fibrillin has two isoforms fibrillin-1 (FBN-1) and fibrillin-2 (FBN-2). The gene for FBN-1 is found on chromosome 15q15-21 and the gene for FBN-2 is found on chromosome 5q23-31 (Lee *et al*., 1991). FBN-1 is a large glycoprotein. It provides strength and is the predominant fibrillin found in the ciliary zonules. FBN-2 provides elasticity. It is not known whether both types of fibrillin can occur together in the same microfibre (Ashworth *et al*., 2000).

Defects in microfibrils as a result of mutations of FBN-1 leads to a wide range of disease phenotypes associated with MFS, from ectopia lentis (EL) to life-threatening neonatal. EL is a major ocular finding in MFS (Kara *et al*., 2011), it occurs in approximately 60% of MFS patients and can also occur in patients who have Marfanoid features (tall with arachnodactyly) but do not fulfil the MFS clinical criteria (Ashworth *et al*., 2000). Table 4 demonstrates the range of ocular conditions associated with both MFS and familial EL, including myopia, cataract, strabismus, glaucoma, corneal flattening and hypoplasia of the ciliary muscle and iris (Cross and Jenson, 1973). The ocular conditions associated with MFS and their influence on IOP measurement using the rebound tonometer will be discussed further in chapter 4.

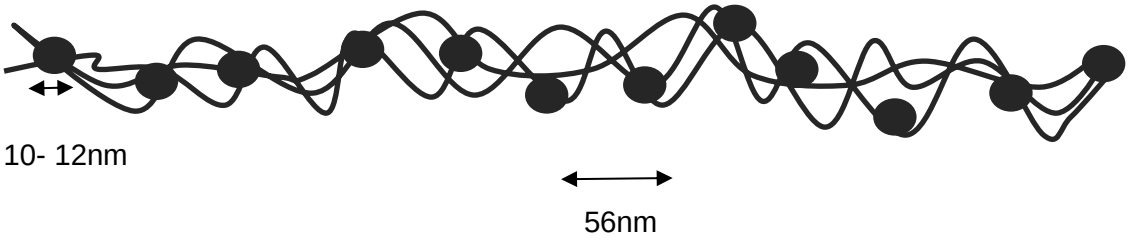


Fig. 9. *Diagram of the structure of a microfibril containing fibrillin-1 (FBN-1), demonstrating the bead like appearance with a diameter of 10 – 12 nm. When the microfibril is relaxed the approximate inter bead distance is 56 nm and this distance increases with tension (Ashworth et al., 2000).*

FBN-1 consists of repeats of tandem calcium binding epidermal growth factor-like (EGF) domains and transforming growth factor β protein-like (TGF- β) domain (Handford, 2000). Mutations of FBN-1 result in elevated TGF- β which affects embryonic development leading to fragile microfibrils. Calcium binds and strengthens the structure of microfibrils. FBN-1 mutation can result in poor calcium binding resulting in weakened microfibrils, which can affect many of the structures of the anterior chamber including the cornea, the lens zonules and the trabecular meshwork. This has been found in a family with familial EL (Ashworth *et al*., 2000, Handford, 2000).

Ehlers Danlos Syndrome

EDS is a collagen disorder caused by mutations of the genes that encode fibrillary collagens or enzymes that aid the building of them. Types I, II, III, V and XI are the main constituent of fibrillary collagens which are proteins that give structure and strength to the extracellular

matrix (De Paepe and Malfait, 2012). EDS is the most common type of HCTD with a prevalence of 1:5000 to 1:10,000 (Rombaut *et al*., 2010). The genetic defects give rise to a range of disease from mild to severe (Tinkle *et al*., 2009).

Twenty-nine different types of collagen have been identified, each containing 3 intertwined alpha chains which make up the triple helix (Fig. 10). The alpha chains are made up of G-X-Y amino acid repeats (Fig. 11). Collagens can be divided into three groups, fibrillary, non-fibrillar and fibril-associated collagens with interrupted triple helices (FACIT). Fibrillary collagens are the largest group found in the body (Jobling *et al*., 2014).

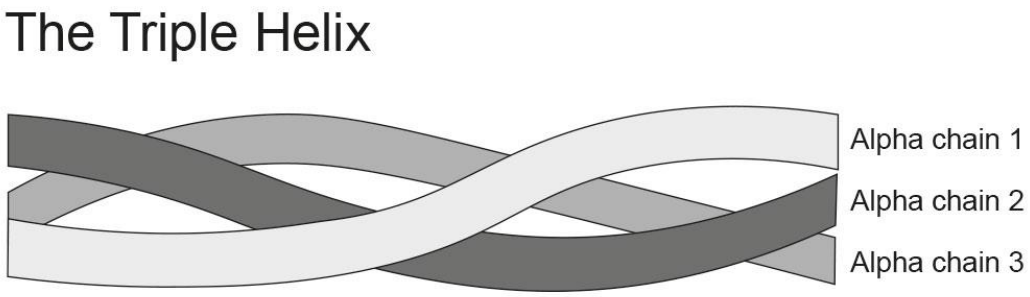


Fig.10. *Diagram demonstrating the intertwined alpha chains that make up the triple helix of collagen.*

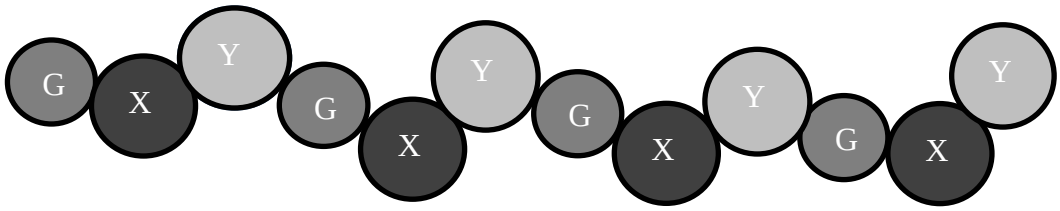


Fig.11. *The alpha chain comprising of G-X-Y amino acid repeats. Glycine (G) is found at the centre of the helix as it is a small amino acid and the X and Y amino acids vary giving rise to different types of collagen.*

The Villefranche Nosology identified six subtypes of EDS consisting of classic (type V collagen defect), vascular (type III collagen defect), dermatosparaxis, kyphoscoliosis, arthrochalasis (all type 1 collagen defects) and hypermobile EDS (genetic basis unknown) with major and minor diagnostic criteria (Table 6). Advances in sequencing lead to the discovery of new variants of EDS. Therefore, in 2017 the International Consortium on the Ehlers-Danlos Syndromes revised the Villefranche Nosology and proposed a new nosology with 13 subtypes (Table 7). They kept the same nomenclature as the Villefranche nosology but added 7 more subtypes and included the major and minor criteria (Malfait *et al*., 2017).

Major criteria
Skin hyperextensibility and atrophic scarring Generalized joint hypermobility
Minor criteria
Easy bruising Fragile skin Molluscoid pseudotumors Subcutaneous spheroids Hernia Epicanthal folds Hypermobile joints/sprains/dislocations 1st degree relative with EDS

Table 6. *Major and minor criteria used in the diagnosis of EDS: adapted from Tinkle et al., (2009.) The presence of one or more major criteria has a high diagnostic value and further molecular testing is used to confirm the diagnosis. Minor criteria by themselves are less suggestive of disease (Beighton et al., 1998).*

The Beighton score is used to diagnose hEDS which is characterised by generalized joint hypermobility, a soft velvety skin and a familial trait (Tinkle *et al*., 2009). The International Consortium on EDS proposed that generalized joint hypermobility (GJH) is present with a Beighton score of ≥6 out of 9 points in pre-pubertal children and adolescents (Rombaut *et al*., 2010). GJH is diagnosed with lower scores in adults due to the reduction in laxity with age. Ninety percent of hEDS patients are female and symptoms include: large and small joint hyperextension and dislocation, skin hyperextensibility, arthritis may start at an early age and cardiac problems may occur due to mitral valve prolapse leading to pain, chronic disability and increased morbidity (Rombaut *et al*., 2010). Ocular complications vary according to the subtype of EDS and will be discussed further in chapter 4, (Table 7).

EDS Subtype	Autosomal Inheritance	Molecular basis of defect
Classical (cEDS)	Dominant	Type I & V collagen
Classic-like (cIEDS)	Recessive	Tenancin XB
Cardiac-valvular (CVEDS)	Recessive	Type I collagen
Vascular (vEDS)	Dominant	Type I & III collagen
Hypermobile (hEDS)	Dominant	Unknown
Arthrochalasia (aEDS)	Dominant	Type I collagen
Dermatosparaxis (dEDS)	Recessive	ADAMTS-2
Kyphoscolic (kEDS)	Recessive	LH1, FKBP22
Brittle cornea syndrome (BCS)	Recessive	ZNF469, PRDM5
Spondylodyplastic (spEDS)	Recessive	β4GalT7, β3 GalT6, ZIP13
Musclocontractural (mcEDS)	Dominant	D4ST1, DSE
Myopathic (mEDS)	Dominant & recessive	Type XII collagen
Peridontal (pEDS)	Dominant	C1r, C1s

Table 7. *2017 International Classification of EDS: adapted from Malfait et al., 2017. Hypermobile EDS (hEDS) is the most common type of EDS (Beighton et al., 1998). It can be difficult to diagnose because molecular testing used for other types of EDS is not useful and it can be confused with benign hypermobility syndrome (BJHS) (Rombaut et al., 2010).*

Osteogenesis imperfecta (OI)

The primary collagen in bone is type I, which is secreted by osteoblasts and mineralizes to form bones as they grow and remodel (Van Dijk *et al*., 2010). Defects in type I collagen leads to OI and is characterized by osteopenia and bone fragility. Patients with OI tend to be short with progressive deafness, abnormal teeth, lax ligaments and blue sclera (Jobling *et al*., 2014). The prevalence of OI is 6-7/100,000 and the severity of the disease is highly variable from mild to lethal (Van Dijk *et al*., 2010). In 1979 OI was classified into four subtypes by Dr David Sillence and were known as the Sillence classification. In 2004, this was subsequently expanded into seven subtypes based on distinct clinical and histological differences. Most patients with OI have known or presumed mutations to COLA1 and COLA2 type I collagens (Rauch and Glorieux, 2004).

Benign joint hypermobility syndrome (BJHS)

BJHS is a common HCTD and often goes undiagnosed. Patients may present with joint laxity with associated dislocation, subluxation and pain (Jacob and Grubb, 2012). Diagnosis is made from a combination of symptoms and objective findings classed as major and minor criteria. As with other HCTDs the Beighton score can be used for diagnosis. Arthralgia (joint pain) is considered to be one of the major criteria for BJHS (Remvig *et al*., 2014).

Stickler syndrome

Stickler syndrome is caused by mutations to types II, IX and XI collagen and is subtyped according to the affected gene and inheritance pattern (Jobling *et al*., 2014). Babies with Stickler syndrome may be born with a cleft palate and Pierre Robin sequence (small lower jaw, backwards displaced tongue and obstruction of the airways). There may also be mild joint hypermobility from which early osteoarthritis can develop. Ocular findings of Stickler syndrome include high myopia, retinal detachment and congenital cataract (Snead and Yates, 1999).

1.8 Instruments

Since the 16th century a firmness of the eye has been associated with certain types of blindness (Brandt and Roberts, 2014). However, it was not until the late 19th century that accurate attempts were made to measure IOP with the invention of the Malakoff applanation tonometer (Stamper, 2011).

In this section a brief overview of some of the instruments that are used to measure IOP is given, with special reference to the rebound and Goldmann tonometers used by the present study. An understanding of the different types of tonometers and their advantages and disavantages leads to an appreciation of the significant impact the rebound tonometer has had when measuring IOP in children.

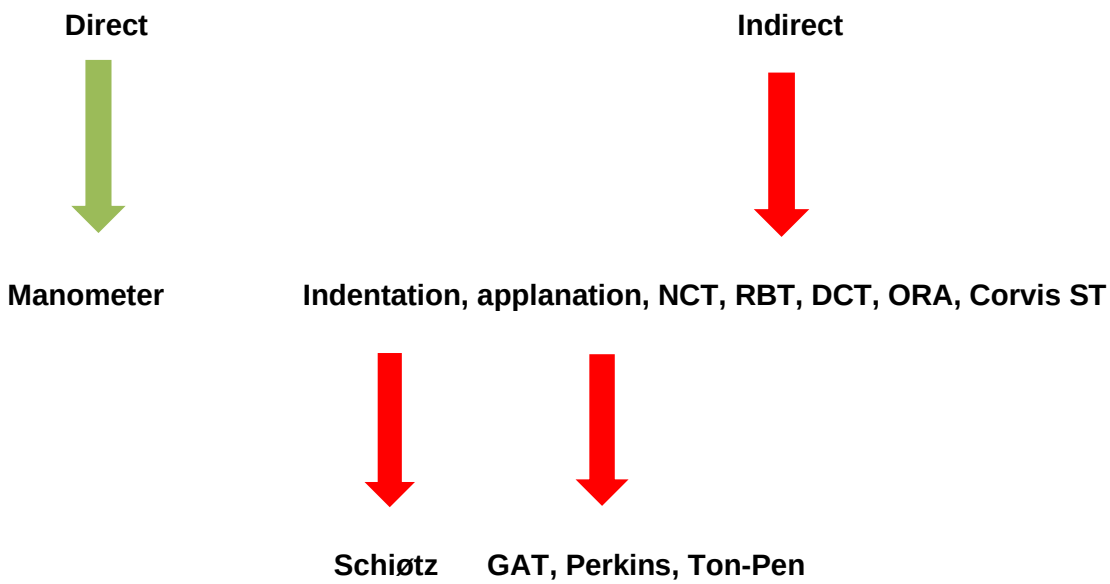


Fig.12. *Different types of tonometers used to measure IOP. The manometer is the only direct way to measure true IOP, but is primarily used in research due to its invasive nature. Tonometers used in hospital eye departments and optometric practices use indirect methods.*

1.8.1 Manometer

Manometry measures the true IOP of the eye because it is a direct measurement and is therefore not affected by corneal biomechanics (Eisenburg *et al*., 1998). Fig. 13 demonstrates the principles of manometry, where the aqueous escaping from the eye is prevented from doing so by the fluid in the manometer, leading to a measurement. Due to its invasive nature, manometry it is only used to measure IOP for research purposes using enucleated eyes and patients undergoing eye surgery (Ellingsen and Grant, 1971; Blumenthal *et al*., 1992). Several manometry studies have looked at IOPs in enucleated eyes; however, post-mortem changes have affected the results and there are few studies on live healthy eyes (Pallikaris *et al*., 2005). The Ton-Pen compares well with the manometer however applanation tonometry underestimates manometric IOP in young children with the difference increasing as the age of the child decreases (Eisenburg *et al*., 1998).

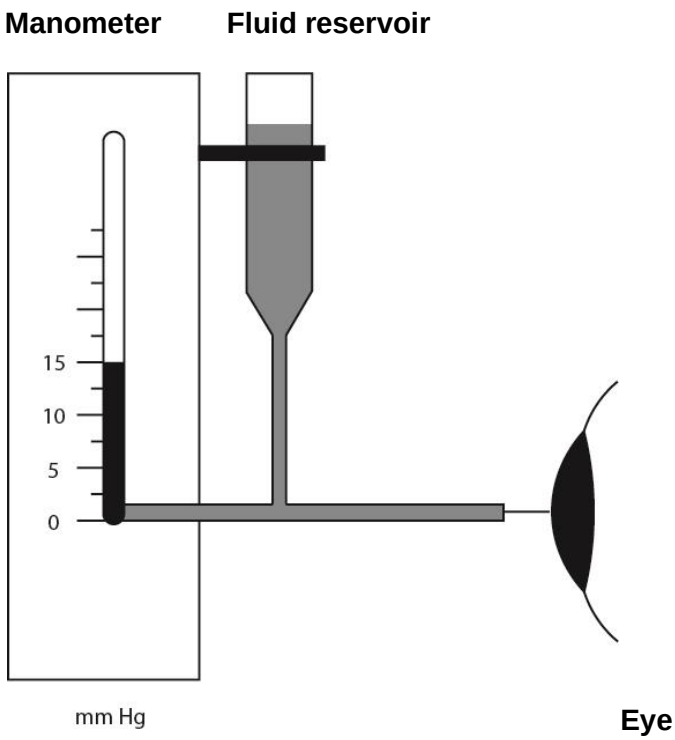


Fig.13. *A schematic diagram demonstrating the principles of manometry. Following anaesthesia, a needle is inserted directly into the anterior chamber of the eye. As the IOP is higher than atmospheric pressure, aqueous fluid will tend to flow out of the eye. The reservoir is raised until fluid loss from the eye is prevented; the height of the fluid in the reservoir relates directly to the IOP in mmHg (Kniestedt et al., 2008). The cannula is connected to a manometer (pressure transducer) which registers the pressure.*

1.8.2 Schiøtz tonometry (ST)

The Schiøtz tonometer is an indentation tonometer that was used worldwide in the early to middle 20th century (Stamper, 2011). It is easy to use, cheap, portable and good for community screening, especially in developing countries (Krieglestein and Waller, 1975; Nagarajan *et al*., 2016).

Measurements are taken with patients in the supine position. Following local anaesthesia, the patient is asked to look straight upwards and fix on a target. The eyelids are held open by the operator without exerting pressure on the eye and the footplate of the tonometer together with a weight is placed onto the cornea. The IOP is then calculated from the scale, with different weights used according to the IOP (Nagarajan *et al*., 2016).

Schiotz tonometry is affected by corneal curvature and scleral rigidity, which differs between children and adults (Youn *et al*.,1990). Myopic eyes have lower scleral rigidity than emmetropes which can lead to underestimation of IOP; hyperopes and scarred corneas have higher rigidity and can result in over estimation (Kniestedt *et al*., 2008).

1.8.3 Tono-Pen

The Tono-Pen XL (Reichert Ophthalmic Instruments, Buffalo, USA) developed from the Mackay-Marg principle of measuring IOP (Nessim *et al*., 2013). The tip of the Mackay-Marg tonometer is pressure sensitive and records the force with which a defined area of cornea is flattened. A plate that surrounds the tip comes into contact with the cornea and repels the surrounding corneal structural resistance; it is at this point that the true IOP is recorded and calculated using the Imbert-Flick rule, Pressure (IOP) = Force/Area, (Stepanik, 1970). The Tono-Pen is a miniature version of the Mackay-Marg tonometer (Hessemer et al., 1989). It has a 1.2 mm tip connected to a strain sensor, which is surrounded by a 3.2 mm collar. Before measurement a local anaesthetic is instilled to numb the front of the eye and the tip of the probe is covered by a latex sleeve. Following calibration, if necessary, the patient is asked to look straight ahead whilst the tip of the probe applanates the centre of the cornea (Lester et al., 2001). Several measurements are taken and a microprocessor analyses the waveforms produced by the sensor producing a digital IOP display (Azuara-Blanco *et al*., 1998). A single line indicates that a reading is reliable (Lester et al., 2001). The Tono-Pen can be used during eye surgery and on bedridden patients in community practice as it is small handheld and can be used in any position (Van der Jagt and Jansonius, 2005).

The Tono-Pen is a type of applanation tonometer however the applanation area of the cornea is 2.26 mm^2 which is much smaller than GAT which is 7.35 mm^2. Therefore, it has been argued that the Tono-Pen is less affected by CCT and tear film (Sulllivan-Mee and Pham, 2004; Chihara, 2008). Although Kniestedt *et al*. (2008) point out that as is the case with other applanation tonmeters the Tono-Pen it is affected somewhat by CCT. It has disposable tips to prevent cross-infection, is small, portable, battery operated, easy to use, can be used in any position and can be used on corneas with poor integrity; however, it does need to be calibrated (Azuara-Blanco *et al*.,1998; Onochie *et al*., 2016).

A study by Horowitz *et al*. (2004) found that Tono-Pen underestimated GAT by an insignificant mean difference of –0.41 mmHg (SD: 2.59) for IOPs < 25 mmHg. For higher IOPs >25 mmHg, Tono-Pen significantly underestimated GAT by a larger mean difference of – 4.2mmHg ($p = 0.0004$), SD:4.6 with a wide 95% limits of agreement of –13.2 to 4.8mmHg. A good agreement between Tono-Pen and GAT has been found (Tonnu *et al*., 2005, Kato *et al*., 2018). However,

it is recommended that Ton-Pen is not used instead of GAT for IOP measurement (Horowitz *et al*., 2004).

Tono-Pen underestimates applanation tonometry (Perkins) in children's enucleated eyes and this difference increases with higher IOPs. However, Eisenberg *et al*., 1998 found that in vivo TP overestimates in children's eyes at low IOP and underestimates at high IOP. They did not state what the meant by higher and lower IOPs, but examination of a graph in their paper suggests this may be IOPs above and below 15 mmHg.

García-Resúa *et al*. (2006) in their study of 68 adult participants found the Tono-Pen XL compared well with the Icare rebound tonometer with both instruments having similar 95% levels of agreement compared with the hand held Perkins tonometer (Icare, −7.81 to+1.12 mm Hg; Tono-Pen XL, −7.74 to +2.18 mm Hg).

1.8.4 Non-contact tonometer (NCT)

The NCT was first introduced by Grolmar in 1972. The original version was table mounted, large, heavy and expensive; however, its main advantage was that it did not need a local anaesthetic as it did not come into contact with the eye, avoided cross-infection and was good for screening (Shields, 1980). The original NCT (Reichert Ophthalmic Instruments, Depew, New York) measures the time taken for the nonlinearly increasing air puff to applanate the cornea; usually 1 to 3 milliseconds and calculates the IOP from this. The time taken to flatten the cornea increases with IOP and corneal rigidity (Shields, 1980). Several measurements are taken due to the effect of the cardiac rhythm, until 3 measurements within 3 mmHg of each other are obtained (Myers *et al*., 1975). Shields (1980) found a good agreement between the original NCT and Goldmann, except at high IOPs. Kouchaki *et al*. (2017) also found a good agreement between the Keeler Pulsair and GAT ($p < 0.001$, Pearson correlation coefficient = 0.820).

Fig.14. *The Keeler Pulsair IntelliPuff tonometer (PT00) (*Keeler Ltd, Windsor,UK) *can be used in the supine position. It is small, portable and easy to use. Therefore, it can be used by non- ophthalmic colleagues (Vernon et al. 1990).*

The Keeler Pulsair tonometer (Keeler Ltd Clewer Hill Road, Windsor, UK) is a newer and popular form of NCT and is used widely in optometric practice and hospital Eye Departments. It is small and portable and measures the magnitude of the air pulse needed to applanate the cornea when aligned correctly. Its main advantage is that it can be used in the supine position (Fig. 14) and as a result, is a useful method of IOP measurement during surgery (Evans and Wishart,1992). Hubanova *et al*. (2015) found a good agreement between the Keeler Pulsair IntelliPuff tonometer and GAT in participants at both normal and high IOPs. However, the Intelipuff tended to overestimate IOPs when compared with GAT at all IOPs. Similarly, Tonnu *et al*. (2005) found that in participants with ocular hypertension and glaucoma, the Canon TX-10 NCT (Canon USA Inc, One Cannon Plaza, Lake Success, NY, USA) overestimated GAT at high IOPs and underestimated at low IOPs. Yildiz and Yasar (2018) found a good agreement between NCT (Keeler Pulsair, USA) and GAT and recommend that NCT can be used instead of GAT. Kontiola *et al*. (2004) compared the Pulair 3000 tonometer with the Icare rebound tonometer in an elderly population and found a difference of < 2 mmHg in 71.7 % participants. Just as with GAT the NCT is affected by CCT and corneal rigidity. However, it is less affected by the tear film (Chihara, 2008).

1.8.5 Ocular response analyser (ORA) and Corvis ST

The ORA (Reichert Inc., Depew, NY) measures corneal biomechanical factors in vivo and IOP independent of the influence of these factors. Corneal biomechanical factors are calculated by observing the corneal response to a high-speed stream of air (Medeiros and Weinreb, 2006). The ORA delivers a pulse of air to the cornea in a similar way to the non-contact tonometer (NCT) which results in mild concavity and the first IOP is measured. As the cornea returns to a normal shape, a second IOP is measured, all taking place within 20 milliseconds (Luce,2005). Corneal deformation is recorded by an infrared light sensor, which characteristically shows as two peaks relating to the first and second IOP measurements (Jędzierowska and Koprowski, 2019). The viscoelastic characteristics of the cornea, e.g rigidity affect the first IOP measurement by resisting corneal deformation, therefore the first and second IOP measurements are not the same (Kouchaki *et al*., 2017). CH is an indicator of viscous damping due to the cornea absorbing and dissipating energy and is calculated from the difference between the first and second IOP measurements (Luce, 2005; Martinez-de-la Casa *et al*., 2006). However, McMonnies (2012) suggests that the CH derived from the ORA may not be the true CH but rather the “central applanation-derived hysteresis” due to variables such as corneal oedema and temperature, location, area and rate of sequencing. The corneal resistance factor (CRF) is also calculated using the CH together with a coefficient (Kouchaki *et al*., 2017).

Lam *et al*. (2007) found a good agreement between the ORA and GAT in a healthy Chinese population with mean ORA slightly higher than GAT (95% CI = 4.55 to -4.44 mm Hg). Cooperation in children is better with ORA than GAT. However, GAT is a better method of IOP measurement than OCR in the presence of nystagmus (Kirwan *et al*., 2006).

The Corvis ST is an NCT which together with an ultra-fast Scheimpflug camera records corneal deformation following a puff of air, producing a video of the process. This results in a more detailed examination of corneal deformation than the OCR which depends solely on the detection of an infrared light signal (Jędzierowska and Koprowski, 2019). A larger number of quantitative data related to corneal biomechanics is obtained, which can be processed with the aids of artificial intelligence (Glowacz *et al*., 2015).

Both the OCR and the Corvis ST produce corrected IOP values: For the OCR the IOP is independent of the influence of CCT and for the Corvis ST the corrected IOP value is based on an algorithm of previous corneal deformations and age and CCT (Luce, 2005; Joda *et al*., 2016.)

1.8.6 Dynamic contour tonometer (DCT)

The DCT (Pascal DCT, Swiss Microtechnology AG, Port, Switzerland) is one of the newer concepts in IOP measurement and is one of the most accurate tonometers as it is not affected by corneal biomechanical properties (Stamper, 2011). It does not cause corneal distortion and is therefore less affected by corneal curvature and thickness (Kouchaki *et al*., 2017). Nessim *et al*. (2013) found that DCT is not affected by biomechanical factors associated with the cornea in their study of 114 patients with glaucoma, NTG and OHT. However, it does require patient cooperation for precision measurement (Chihara, 2008). A significant correlation between the DCT and GAT ($p < 0.001$, Pearson correlation coefficient = 0.812) has been demonstrated by Kouchaki *et al*. (2017). However, the mean IOP measured by the DCR was significantly higher than GAT by 1.6, SD 2.1, $p < 0.001$.

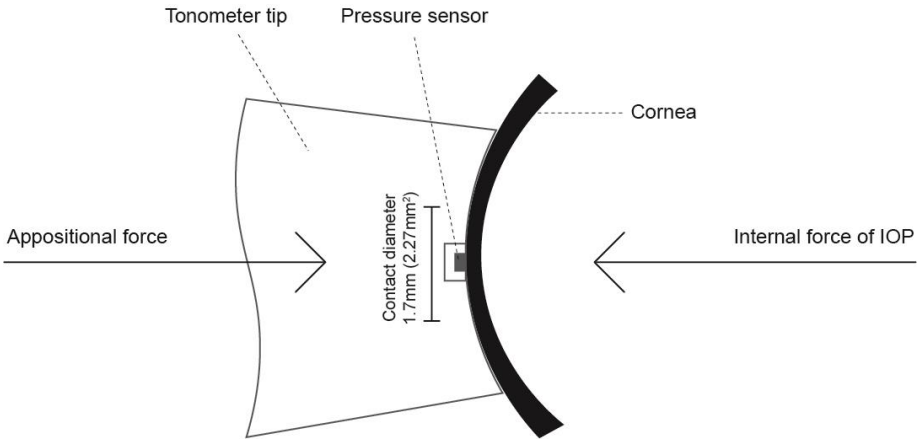


Fig.15. *The tonometer head of the DCR. It is a digital contact tonometer which has a concave surface with a radius of curvature of 10.5 mm and diameter of 7 mm. At the centre of the tip there is a sensor which measures the trans-corneal IOP when the tip of the tonometer head fits the cornea (Adapted from Kouchaki et al., 2017).*

1.8.7 Goldmann tonometry

The Goldmann applanation tonometer (GAT) is considered to be the gold standard method of measuring IOP (Kniestedt *et al*., 2008) and is currently the most popular tonometer used by ophthalmologists (Cook *et al*., 2012). GAT is a fixed area applanation tonometer that was first used in 1957 (Dielmans *et al*.,1994). Applanation tonometry is based on Hans Goldmanns Imbert Fick law that "the pressure in a sphere filled with liquid and surrounded by an infinitely thin membrane is measured by the counter pressure which just flattens the membrane." This law, in turn, derives from Newton's third law regarding pressure. When applied to the eye this law becomes "if you press the eye with a tonometer, the tonometer is pressed by the eye" (Mark, 2012). Therefore, Pressure = Force applied/area applanated.

A plastic cone with a total area of 7mm and a tip with an area of 3.06 mm is used to applanate the cornea. The rigidity of the cornea is counter balanced by the surface tension of the tears and assuming a cornea with a central corneal thickness of 0.5 mm, a force of $1/10^{th}$ gm on the circular area of the tip is equal to 1mmHg (Kniestedt *et al*., 2008). The application of the cone onto the cornea displaces 0.5 µl of aqueous fluid which raises the IOP by 3%, however due to the elasticity of the eye this does not significantly affect the final IOP reading (Stamper *et al*., 2009a). Two types of GAT are available; one that is permanently mounted on the slit lamp (R type) and another (T type) that is mounted on a removable plate (College of Optometrists, accessed 30/11/2019). The Perkins handheld applanation tonometer is a portable applanation tonometer (Fig. 18).

Fig.16. *The T type D-KAT Goldmann applanation tonometer (Keeler UK) used by the present study, mounted on a slit lamp.*

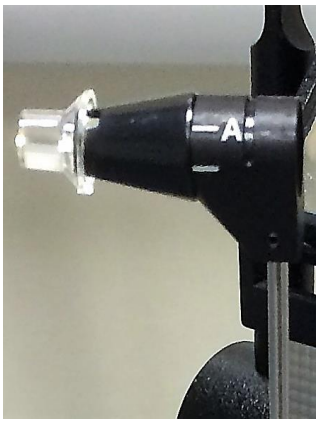


Fig.17. *Tonometer head with disposable plastic probe as used by the present study (Haig Streit, Harlow, UK)*

Fig.18. *The Perkins Handheld applanation tonometer (Haag-Streit, Harlow, UK).*

1.8.7.1 Taking eye pressure measurements

The patient is seated at the slit lamp bimicroscope with his/her head resting on the chin rest and the forehead against the forehead rest. The GAT is fitted with a sterilized or disposable probe. A cobalt blue filter (wavelength 390nm to 410nm) on the slit lamp is selected (Dielemans *et al*., 1994). The slit beam is maximized and placed so that the angle between the illumination and the microscope is approximately 60° thus fully illuminating the probe and the surrounding room illumination should be low (Stamper *et al*., 2009a).

One drop of topical anaesthetic and fluorescein are instilled into the lower fornix of the eye to be measured. Fluorescein is a dye that fluoresces as a bright green/yellow light when illuminated by cobalt blue light. The plastic probe of the GAT gently applanates the centre of the cornea creating a tear meniscus which is seen when looking through the centre of the probe. Two prisms situated within the probe split the meniscus into two separate semicircles. The force on the probe is increased by turning the tonometer knob until an area with a diameter of 3.06 mm is applanated. At this point the inner parts of the semicircles touch and a reading is taken. In some cases, the semi-circles pulsate with the pulse and the middle of the pulsation is taken as the result. In cases with high astigmatism of over 3D average readings can be taken over the flat and steep meridians (Kniestedt, 2008).

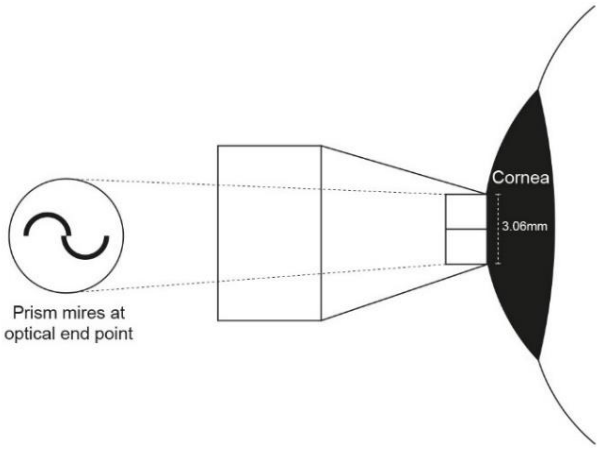


Fig.19. *This demonstrates the action of the GAT tonometer probe and the end point measurement when the inner sides of the semi circles touch.*

1.8.7.2 Advantages and disadvantages

Unlike RBT, GAT must be checked for calibration regularly. Haag-Streit recommend calibration checks are carried out monthly, using a standard weight bar and two independent observers and an acceptable calibration range of ±0.5 mm Hg (Sandhu *et al*., 2005). GAT measurements can be affected by hypofluorescence of the precorneal tear film, CCT, astigmatism, corneal curvature, valsalva movement, eyelid squeezing, vertical gaze and indirect pressure on the globe some of which can be controlled (Brandt and Roberts, 2014; Whiteacre and Stein. 1993). Repeated IOP measurement can cause a reduction of IOP and variation in corneal resistance and oedema can lead to false readings (Whitacre and Stein. 1993). Variation in corneal thickness affects GAT measurements, leading to over and under estimation of IOP (Elsheikh *et al*., 2006). Corneal curvature can also affect GAT readings with steeper curvatures resulting in higher IOPs due to the extra force needed to flatten them. In addition, to produce the required area of applanation more aqueous is displaced from under a steep cornea which increases the ocular rigidity and also leads to higher IOP measurements. A 1 mm increase in the radius of corneal curvature (corneal flattening) can lead to a 3.33 mmHg reduction of IOP (Medeiros and Weinreb. 2006). Nessim *et al*. (2013) found that GAT is affected by corneal biomechanics (CH and CRF). In addition, variations in the tear film produce different surface tensions and intermolecular forces (Chihara, 2008).

The GAT must be mounted on a slit lamp and is therefore not portable and requires the patient to sit upright and be able to place their face correctly onto the chin and forehead rests.

This can be difficult for children the elderly and some obese patients (Kniestedt *et al*., 2008). Anaesthetic eye drops are used to numb the cornea prior to taking measurements. This can cause discomfort and can lower the IOP (Baudouin and Gastaud, 1994). Tonometer probes can be disposable and reusable. Disposable probes prevent cross infection and can be more convenient. Measurements using *Tonosafe*™ disposable probes compare favourably with reusable probes with a mean difference of < 0.5 mmHg, however there is a larger interobserver variability for observers using disposable probes (Ajtony *et al*., 2016). Earlier indentation methods such as the Schiøtz tonometer displaced a large amount of fluid leading to a rise in IOP. GAT displaces an insignificant amount resulting in a more accurate estimate of actual IOP (Moses, 1958). Other tonometers such as the non-contact tonometer (NCT) and the Perkins handheld applanation tonometer (Fig. 18) compare well with GAT (Cook *et al*., 2012). However, due to its mechanical design repeated measurements with GAT are more similar than NCT (Thorburn,1978). The dynamic contour tonometer (DCT) is not affected by corneal thickness and agrees well with GAT, but can read slightly higher (Kaufmann *et al*., 2004). As GAT is influenced by corneal biomechanics and CCT this can lead to measurement errors and misclassification of diseases. After correcting for corneal thickness approximately one third of patients with normal tension glaucoma would be diagnosed with POAG and a half of OHT patients would be normal (Copt *et al*.,1999). However, in spite of these disadvantages GAT is currently the gold standard method of measuring IOP.

1.8.8 Rebound tonometry (RBT)

The Icare Rebound tonometer (Tiolat Oy, Helsinki, Finland) is a non- invasive induction /impact method of measuring IOP (Kontiola, 1997). It is hand held and the TA01i version used for this project is 13 – 32 mm wide, 45 – 80 mm in height and 230 mm long and weighs 250 gm including batteries (users and maintenance manual – Icare tonometer 2009).

Fig. 20. *TA01i Icare tonometer used by the present study. The photograph shows the probe in situ.*

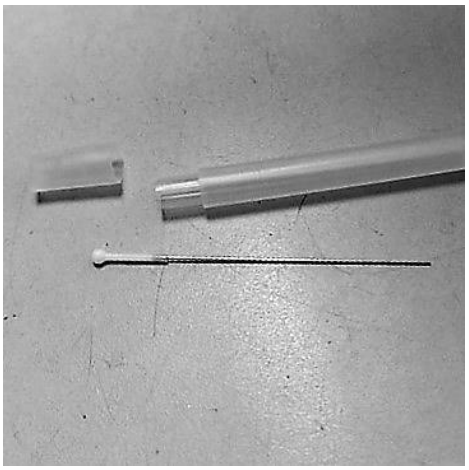


Fig. 21. *TA01i disposable single use magnetic probe 40 mm long, 0.3 mm in diameter with a plastic tip 1.7 mm in diameter and a total mass of 26.5 mg (Davies et al., 2006).*
The probe is sterile and is stored in a plastic case as shown above before use.

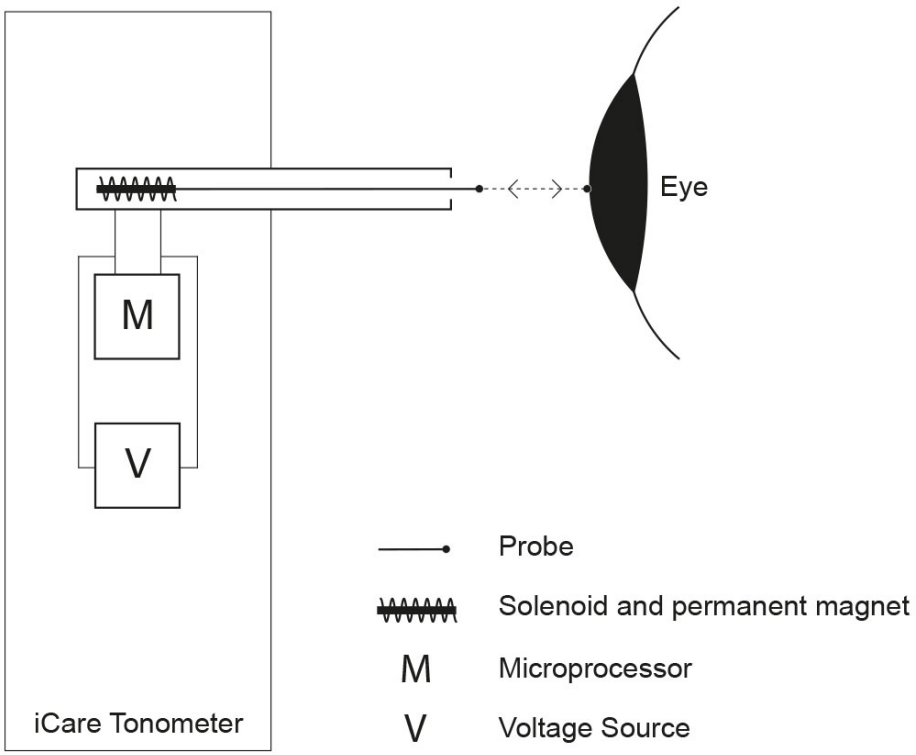
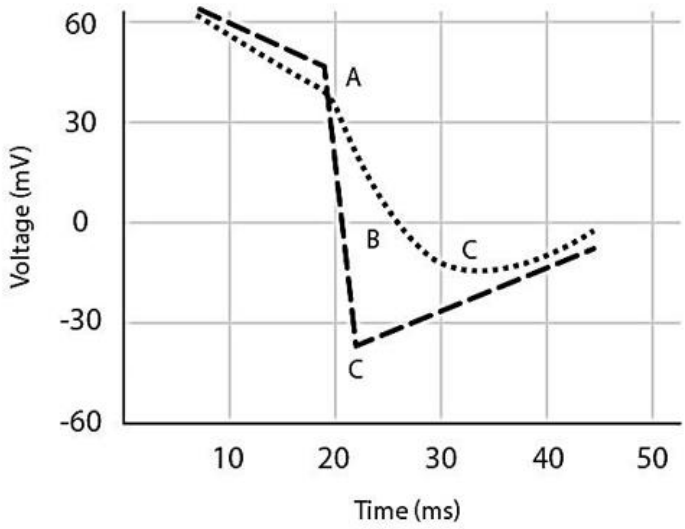


Fig. 22. *Schematic diagram of the measurement system of the iCare device, demonstrating the solenoid with permanent magnet and microprocessor. The probe is propelled over Teflon™ bearings by a short electrical current of 30 milliseconds and travels towards the cornea at a speed of 0.2 metres per second. The microprocessor monitors the movement of the probe and detects the deceleration speed of the probe after it bounces off the cornea thereby calculating the IOP (Davies et al., 2006). The inverse of the probe's deceleration time has been found to correlate well with manometric measurements on enucleated rodent eyes between 5 mmHg and 60 mmHg (Kontiola et al., 2001)*

A lightweight magnetic probe with a plastic tip is propelled towards the cornea by a magnetic force created within a solenoid by a 30 milliseconds current which induces a voltage (Fig. 22). Following impact with the cornea the probe decelerates and rebounds back into the solenoid changing the direction of the voltage. The speed of the probe determines the voltage, which is analysed by a microprocessor. The impact time and change of direction of the probe are also detected. Higher speeds with shorter contact times result in higher readings (Kontiola, 2000) (Fig. 23). Six readings are taken, the highest and lowest are eliminated, the average measurement is calculated from the remaining four readings by inbuilt software (Asrani *et al*., 2011).



A: Probe impacts the eye. B: Probe changes direction. C: Probe leaves the eye
…… 5 mmHg **--------** 40 mmHg

Fig. 23. *Signals obtained at 5 and 40 mmHg demonstrating longer probe corneal contact times and slower deceleration at lower pressures and shorter contact times with faster deceleration at higher pressures. Tests on rodent eyes have shown that the deceleration time of the probe correlates well ($r^2 = 0.95$) with IOP measured in mmHg for pressures from 5 to 60 mmHg: Adapted from Kontiola et al., (2001).*

1.8.8.1 Taking eye pressure measurements

The probe is inserted into the probe base of the Icare tonometer directly from its plastic cover without touching it to avoid contamination. The tonometer is then activated by pressing the measurement button. This magnetizes the probe preventing it from falling out of the probe base. A “00” will appear in the display window when the tonometer is ready to take a measurement. The forehead support can be adjusted and placed on the patient’s forehead to enable a measurement to be taken at the right working distance.

Fig. 24. *Demonstrates the position of the Icare TA01i tonometer when taking a measurement. The probe is held at 90 degrees 4 to 8 mm from the centre of the cornea., with the patient looking straight ahead. The operator takes 6 successive measurements, the instrument discards the highest and lowest and calculates the IOP from the remaining four.*

With the patient looking straight ahead the tonometer is positioned so that the probe is horizontal, the distance from the tip of the probe to the centre of the cornea is 4-8 mm (Fig. 24). The measurement button is lightly pressed resulting in the probe making contact with the cornea. Six consecutive readings are taken with a short beep sounding with each one. A final longer beep will signify the end of the measurement. A “P” will be seen in the display window followed by the IOP reading. If “P” is flashing then the standard deviation of the reading is greater than normal. Lines next to the “P” indicate the level of the deviation of the standard deviation (users and maintenance manual – icare tonometer) (Fig. 25). Fortunately, although the speed of the probe is low, it is faster than the corneal blink reflex (Kontiola, 2000).

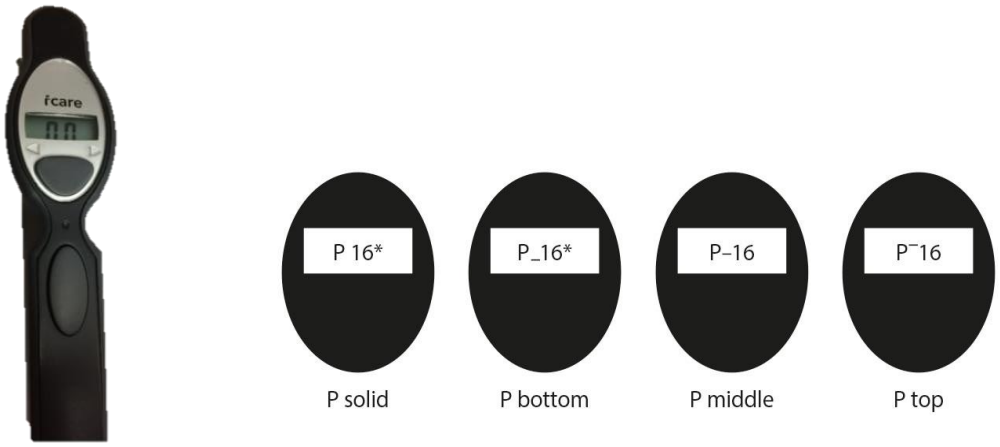


Fig. 25. *The TA10i Icare tonometer demonstrating the different levels of reliability: P solid (SD < 1.8 mmHg) is the most reliable. A flashing P with a line at the bottom is the next most reliable (SD 1.8 to 2.5 mmHg). P middle (SD 2.5 to 3.5 mmHg) is acceptable for IOPs < 20 mmHg. P top (SD > 3.5 mmHg is considered to be an unreliable reading and should be disregarded (Lambert et al., 2013).*

Since the production of the TA10i Icare rebound care tonometer in 2003, other types of Icare rebound tonometers have been produced in response to the need for supine measurements and patient self-monitoring. The Icare PRO, released in 2010, has an inclination sensor to prevent the probe from falling out when used in the supine position and has a good correlation and agreement with the hand held Perkins applanation tonometer, which can also be used in a similar way (Jabolinski *et al*., 2013). Following this, the Icare ONE was produced to enable self-monitoring of IOP at home. The Icare One is easy to use and has a good correlation with GAT, but has been found to underestimate when compared with GAT for IOPs between 10 and 20 mmHg (Rosentrater *et al*. (2011). In 2014 a newer version of the Icare One was released. The Icare HOME has an eye recognition system and position monitor and is similar to the Icare One. It has also been found to underestimate GAT (Cvenkel *et al*., 2019). In 2016 an updated version of the original Icare TA01i, was produced. The Icare ic100 has a horizontal position sensor and rapid measurement facility which can result in more accurate measurements and has a good correlation with GAT except at IOPs <10 and > 24 mm Hg (Wong et al., 2018). An Intra Class Coefficient (ICC) of 0.77 (95% CI of 0.71 - 0.82 mmHg) was found by, Wong *et al*., (2018) which was a good indicator of reliability. The TA01i can be easier to use on children than the new ic100 because the ic100 is position sensitive and children may not keep still during measurement (Nakakura, 2018). The author of the present study has experience with both instruments and can confirm this observation.

1.8.8.2 Advantages and disadvantages comparison with GAT

The Icare rebound tonometer is light, portable, reliable, does not require regular calibration checks and its single use probe reduces the risk of microbial transmission (Lambert *et al*., 2013). It does not require the instillation of a local anaesthetic and is easy to use even by inexperienced clinicians (Abraham *et al*., 2008). Intra-user reproducibility between experienced technicians and newly trained users has been found to be good (Asrani *et al*., 2011). School children tolerate RBT well with high intraobsever and interobserver correlation (Sahin *et al*., 2007). The Icare tonometer can be used by family members to measure the IOP of their child at home in a more familiar environment with minimal risk and can be used multiple times to look at diurnal variation (Flemmons, 2011a). IOP measurement can be difficult in children and infants who do not tolerate GAT or NCT well and in such cases examination under anaesthesia (EUA) may be used to enable measurement (Borrego Sanz *et al*., 2016). This is not ideal due to the risks involved and in addition, general anaesthetics can increase aqueous outflow resulting in reduction of IOP leading to an underestimation of measurements (Murphy, 1985). Infants have been shown to tolerate RBT well (Lundvall *et al*., 2011) which has led to a significant reduction in the use of EUAs in paediatric glaucoma patients (Grigorian *et al*., 2012). On the whole children under the age of six tolerate RBT better than the non-contact tonometer (Kageyama *et al*., 2011).

RBT readings are significantly affected by central corneal thickness (CCT). A 10% increase in CCT in adults can lead to a 9.9% increase in RBT results and the difference between RBT and GAT increases significantly with increasing CCT (Poostchi *et al*., 2009). CH and CRF also influence RBT readings substantially (Chui *et al*., 2008) which has been discussed in section 1.6.3.

Several studies looking at the agreement between RBT and GAT in adults have found good overall concordance (Brusini *et al*., 2006; Schreiber *et al*., 2007; Johannesson *et al*., 2008, Kato *et al*., 2018). Experienced tonometrists can obtain RBT readings ≤ 2 mmHg of GAT 80% of the time (Abraham *et al*., 2008). RBT has a small but not significant positive bias compared with GAT (Davies *et al*., 2006; Scuderi *et al*., 2010). Intersessional reproducibility of RBT is not quite as good as GAT but is similar to other methods of IOP measurement (Davies *et al*., 2006). However, not all studies have found a good agreement; a significant difference

between GAT and RBT in adults has been reported with RBT tending to overestimate IOP in adults (Poostchi *et al*., 2009).

Few studies have compared RBT with GAT in children. A comparison of RBT and GAT in children with known and suspect glaucoma found 63% of RBT measurements to be within 3mmHg of GAT (Flemmons *et al*., 2011b). Gandhi *et al*. (2012) have had similar results and also found that where the difference was greater than 3 mmHg, RBT was higher than GAT in 84% of cases. They also noted that RBT readings decrease with repeated sequential measurements as the patient relaxes.

1.8.8.3 Suboptimal measurement

RBT measurements are normally taken at the centre of the cornea with the probe held at a right angle to the cornea (Muttuvelu *et al*., 2012). Young children do not always cooperate well and may look upwards during measurement due to Bell's phenomenon (Francis and Loughead, 1984). Therefore, some readings are taken from other more peripheral areas of the cornea Very few studies have looked at the effect of sub optimal measurements taken from other areas of the human cornea.

Beasley *et al*. (2013) did not find any significant difference for readings taken within 2 mm of the centre of the cornea in adults. They also found that a slightly tilted probe did not affect the measurements. Muttuvelu *et al*. (2012) found that readings taken 2mm from the temporal limbus of the cornea in adults were significantly lower by 3 – 4 mmHg than those taken at the centre. Measurements taken from the sclera have no relationship to GAT readings (Poostchi *et al*., 2009). There is a paucity of information about this in children so this study looks at the reliability and validity of sub optimal off- axis RBT readings in this cohort.

1.9 Summary

Children's glaucoma differs from the adult form in many ways from its origins, clinical findings, severity and the number of co-existing conditions that may be present or associated with it. Failure of the development of the anterior chamber before birth due to faulty gene transcription and the associated syndromes have been described. In the older child JOAG, uveitic, aphakic and pseudophakic glaucoma can occur. IOP measurement by GAT can be

difficult in children with glaucoma due to lack of cooperation and the presence of microcornea, KP, corneal scars, nystagmus, strabismus etc. That is the reason why RBT with its ease of use, versatility, small footprint and non-invasive nature has been adopted so widely by hospital eye departments and optometric practices.

Children with HCTDs such as MFS and EDS can also develop glaucoma and often need to have their IOPs measured during hospital visits. These syndromes develop as a result of collagen defects which can affect the structure of the eye including the trabecular meshwork and the cornea. The defective collagen can affect the corneal structure leading to changes in curvature, thickness and CH all of which can affect IOP measurement.

The instrument section gave a brief insight into the development and challenges of measuring IOP. GAT is currently the gold standard method of IOP measurement, but it is not without its own limitations as it is affected by CCT, CC and CH. RBT has also been shown to be affected by CCT and CH. However, its main advantage is that unlike GAT, it does not require a local anaesthetic and can be used on very young children and babies without an EUA. Previous studies by Flemmons *et al*. (2011b) and Dahlmann-Noor *et al*. (2013) have pointed out that not enough is known about the reliability of RBT in children with glaucoma and as far as the author is aware nothing is known about the reliability of suboptimal RBT measurements in children with glaucoma and HCTDs. Therefore, the present study has been designed to address all of the above.

Chapter 2

The validity and reliability of the Rebound Tonometer

2.1 Introduction

Eye pressure measurement is very important in children who have glaucoma or are at risk of developing it and can be difficult if they are young or uncooperative. At the time of submitting this thesis, little data has been published concerning how many children have IOP measurements every year. A small survey of 144 paediatric ophthalmologists about RBT use in hospitals and community practice in the UK found that RBT has become the most popular method of measurement (Dahlmann-Noor *et al*., 2013). As stated previously GAT is the gold standard method of IOP measurement and there is a paucity of information on the reliability of RBT measurements when compared with GAT in children with glaucoma. As far as the author is aware only one previous study by Flemmons *et al*., (2011b) has examined the relationship between age and the difference between RBT and GAT measurements in children with glaucoma.

To address these questions the first part of Experiment 1 in this chapter will examine the validity of RBT_{on} measurements when compared with GAT. The second part will examine the association of age with the difference between RBT_{on} and GAT.

2.2 Methods

Ethics

The present study was conducted in accordance with the tenets of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. Application for approval was complex, took several months and was made by the author via the Integrated Research Application System. The study was approved by the NHS North West - Liverpool East Research Ethics Committee (REC), the Research and Development department (R&D) of Birmingham Women's and Children's Hospital (BWCH) and the Ethics committee of Aston University (Appendix 4). It should be noted that BWCH was known as Birmingham Children's Hospital (BCH) in 2016 during the preparation for this study. The name changed to BWCH in 2018.

A summary of this research has been prepared by the author and has been published on the Health Research Authority (HRA) and International Standard Randomized Control Trial Number registry websites (ISRCTN, 15954407), (Appendix 1). At the end of the first year, and at the end of the study, progress reports were submitted to the NHS North West - Liverpool East REC (See Appendix 4 for confirmations).

General methods

Following ethical approval, a Master Site File was compiled by the author, which contained all relevant and approved documentation for the study including the Patient Identification Log and the Delegation of Duties Log (Fig. 26). The Site File was stored in a secure location within the Eye Department of BWCH when not in use. The Identification Log was used to record the participant's name and GP details. Participants were allocated an identification number for confidentiality and the Delegation Log used to record the duties of those involved in conducting the research (Appendix 4). In order to conduct research within the NHS, the author received training in Good Clinical Practice (GCP). All of the investigative team provided current CV's and (GCP) certificates. Mr Joseph Abbott (Consultant Paediatric Ophthalmologist) and the author also completed GCP training in taking consent. The author gained an Honorary Contract at BWCH in order to conduct research at the hospital, attended an induction day and completed on line governance courses. In addition, the author was required to receive immunization against hepatitis B by the hospital. Professor Leon Davies (Chief Investigator, Aston University) also gained an Honorary Contract with BCH.

All measurements were undertaken at the Eye Department of BWCH from children attending their routine appointments at Mr Joseph Abbotts' clinic between May 2016 and September 2017. The Eye Department of BWCH is a specialist centre for paediatric glaucoma and has a large catchment area with children and their parents travelling from as far away as Wales and Worcester for treatment. Clinics are held 4 days per week, in the mornings and afternoons. After booking in at reception the children have their vision, binocular status and rebound tonometry measurements taken by an orthoptist before seeing Mr Abbott or one of his colleagues. An optometrist undertakes refraction and contact lens checks where necessary. Approximately 18 children attend each clinic one third of which may have glaucoma. Children

also present with a range of other ophthalmic conditions e.g. colobomas, vernal conjunctivitis, ocular and brain tumours.

Following enrolment age appropriate Patient Information Sheets (PIS) were given to participants and their parents and the contents were explained by Nicola Sabokbar. Assent was obtained from all children whilst informed consent was obtained from parents or person with parental responsibility. Following the study visit a letter was sent to the children's GP to inform him/her of study participation, (Appendix 4). This procedure will be explained in more detail in section 2.2.2.

No financial reimbursement was made for parking fees or transport because the patients were attending their routine appointments. However, children were given a "Certificate of Achievement" to thank them for taking part in the study (Appendix 4).

Following completion of data collection, data lock occurred in January 2018. After the study closed and as promised by the author to the children who took part in the study, a poster with a simplified explanation of the results was displayed in the Eye Department of BWCH, thanking them and their parents for taking part (Appendix 4).

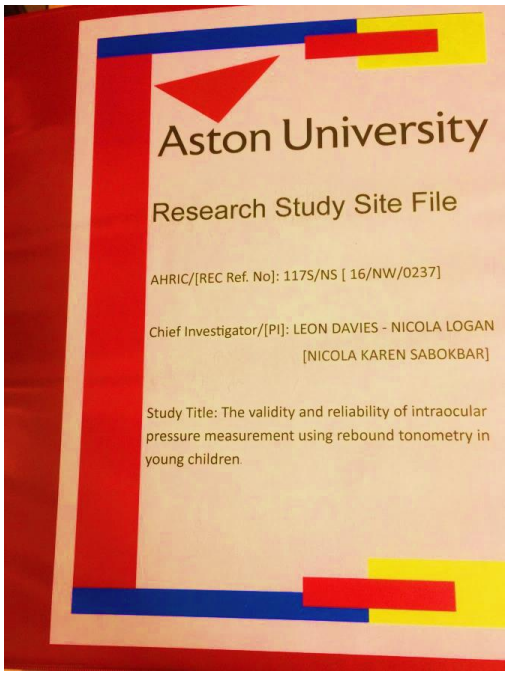


Fig. 26. *The RBT Study Site File contained all the study paperwork including the delegation Log, invitation leaflet, PIS, consent forms, GP notification forms, participant identification Log and completed case report forms (CRF). It was kept in a secure location within the BWCH Eye Department during the study. At the end of data collection, the study was closed and the site file was handed over to Aston University for secure storage. See Appendix 4 for examples of the invitation leaflet, PISs, consent forms, GP notification form, Certificate of Achievement and the CRF.*

2.2.1 Pre-Study Survey

As a part of the preparation for the present study, small pre-study survey was conducted to investigate how well the research would be received by parents and their children attending the Eye Department at BCH. The survey was conducted by one of the healthcare/research team during routine appointments over the course of two days. A survey was needed in order to obtain approval from the Research and Development Department of BWCH.
Following a verbal explanation, the survey was conducted and consisted of the following short questions with yes/no answers:

1. Do you think it is a good idea to compare the new device with the traditional method?
2. The measurements will take a little extra time. Do you think children and their parents will mind?
3. Do you think measurements should be taken during routine eye appointments at the Eye Department?
4. If you were taking part would you prefer to come back another day?

2.2.2 Experiment 1

Part 1

Following assent / consent a total of 34 children with glaucoma (experienced with GAT), aged between one month and sixteen years of any race or gender were recruited from children attending the Eye Department of Birmingham Children's Hospital for routine appointments.
The exclusion criteria for these participants were:

- Participants unwilling to have Goldman Applanation Tonometry and /or rebound tonometry.
- Participants with a known allergy to either proxymetacaine or fluorescein eye drops.
- Pregnancy
- Premature birth (babies < 1 year only)

Suitable participants were identified by the healthcare team of the Eye Department of BCH. An advertisement was also placed in the department waiting room to enable self-referral.

Prospective participants were given an invitation letter and if they were interested in joining the study, Nicola Sabokbar explained the project and what it entailed in more detail. Age PISs were given to the participants and their parents. If they were happy to proceed, assent forms were signed by the children (very young children gave verbal assent) and consent forms were signed by their parents.

Following consent, the child's name and GP details were entered into an identification log and they were allocated an identification number. A case report form (CRF) was then prepared with the patient's identification number and initials.

The RBT readings needed for Experiments 1 and 2 (see 3.2.1) were always taken first. RBT readings were always taken first to eliminate the reduction of IOP due to aqueous displacement by GAT (Fernandes *et al*., 2005). A pseudorandom technique was used for eye selection, i.e. a coin was tossed to decide which eye (right or left) should be used and which reading should be taken first (RBT_{on} or RBT_{off}). However, if only one eye had glaucoma, that eye was chosen (Gandhi *et al*., 2012). Where possible the child was encouraged to toss the coin to encourage participation.

The child's pupillary distance (PD) was then measured and the monocular PD estimated. An Oculus UB 3 Universal Trial Frame (OCULUS Optikgeräte GmbH, Münchholzhäuser Germany) with the monocular PD set at corneal centre or at 3 mm from the centre was then placed onto the child's face and adjusted to fit comfortably (Fig. 27). Beasley *et al*., used a trial frame to align RBT measurements in their 2013 study.

Fig. 27. *A photograph of the Oculus UB 3 Universal Trial Frame (OCULUS Optikgeräte GmbH, Münchholzhäuser Germany) used by the present study to align the RBT probe when taking measurements at the geometric optical centre and 3 mm temporally.*

The Icare RBT tonometer with a fresh probe inserted was aligned with an indicator on the trial frame and six readings were taken or three if the child did not cooperate (Flemmons *et al*., 2011b). All readings except “P top” were accepted and recorded (Flemmons *et al*., 2011b). Following this, the trial frame was removed and the child was prepared for GAT (Fig 28). A drop of Minims® Proxymetacaine Hydrochloride 0.5% (Bausch & Lomb U.K Limited) was instilled into the relevant eye, followed by a drop of fluorescein using BioGlo Fluorescein Strips (Accutome, Inc.) wetted by sterile saline. The child then placed his/her face on the head rest of the slit lamp. Before the GAT reading was taken the integrity of the cornea was checked using the slit lamp (Davies *et al*., 2006). The GAT was then placed onto the slit lamp, the magnification was set to x10, a cobalt blue filter was selected) and the light source was placed at a 60° angle to the GAT probe (Fernandes *et al*., 2005). The GAT digital display was set to 10 mmHg and a fresh probe was gently placed at the centre of the cornea. Once the *Tonosafe*™ disposable probe (Haag-Streit, Harlow, UK) was in the correct position with the semi circles equal in size, the dial on the GAT was moved until the inner part of the semi-circles touched. If the semicircles were pulsing the middle of the pulse was estimated (Dielemans *et al*., 1994). The measurement was then read from the digital display on the tonometer. The corneal integrity was then rechecked post measurement (Davies *et al*., 2006). The RBT and the GAT measurements were taken by either Mr Joseph Abbott or Nicola Sabokbar, both of whom are highly experienced with these instruments. In most cases (97%) both measurements were undertaken by the same observer (Kageyama *et al*., 2011).

Part 2

The participants had consented/assented for additional data to be recorded from their notes. One of these characteristics was their age in years, which was divided into two groups: group 1 (0-9) years, group 2 (10-15) years. The groups were divided this way because it resulted in groups of similar size. GAT readings were then subtracted from RBT_{on} and the resulting values were divided in two different ways. In the first division, the data was divided according the range of IOP and resulted in IOP status A which had two groups (group 1, -5 to 2 mmHg; group 2, 3 to 11 mmHg). The data was also divided into “above” or “below” sub categories as used by previous studies by Flemmons *et al*., 2011b and Dahlmann-Noor *et al*., 2013. This

resulted in IOP status B which was divided into two groups (group 1, ≤ 3 mmHg and group 2, > 3 mmHg).

Fig. 28. *The Haag-Streit slit lamp (Haag-Streit UK Ltd) with T type D-KAT Goldmann applanation tonometer (Keeler UK) used by the present study.*

2.2.3 Statistical analysis

The number of participants needed for each research experiment was calculated using G*Power 3.1.5 software (Franz Faul, Universität Kiel, Germany).

For the first part of Experiment 1 a priori power analysis was selected for a two tailed t-test for the difference between two dependent means with a power of 80%, a Pearson correlation coefficient for medium effect size of 0.5 and an alpha level 5%. This indicated a sample size of 34.

Statistical data were analysed using SPSS for windows and were tested for normality by the Kolmogorov-Smirnov test (K-S test). A probability of <0.05% was taken as statistically significant. Where appropriate, data that were not normally distributed were transformed into Log_10 units and retested for normality.

As the GAT and RBT_{on} values were not normally distributed, the non-parametric Wilcoxon Signed Ranks Test was used to indicate whether there was a useful level of agreement

between the mean of the IOP readings for GAT and RBT_{on} from the 34 children with glaucoma.

Individual differences between GAT and RBT_{on} were calculated by subtracting GAT readings from RBT_{on}. Limits of agreement between transformed GAT and RBT_{on} data (expressed at 95% confidence level, where the mean of the difference +/- 1.96 SD of the differences) were then demonstrated using a Bland Altman scatter plot (Bland & Altman., 1995). This was followed by a linear regression procedure to check for linear trend and proportional bias (agreement between the two measurements).

For the second part of Experiment 1, a Fishers Exact test was used look at the association between age and the difference between RBT_{on} and GAT for IOP status A and status B.

2.3 Results

2.3.1 Survey results

Twenty-one children together with their parents took part in a pre-study survey. 100% of respondents thought that the study was a good idea, 65% did not mind the extra time, 95% preferred to have measurements taken at routine appointments and 71% did not want to come back another day. It should also be noted that 35% of respondents did mind the extra time needed to take part in the study. However, 29% were prepared to return another day.

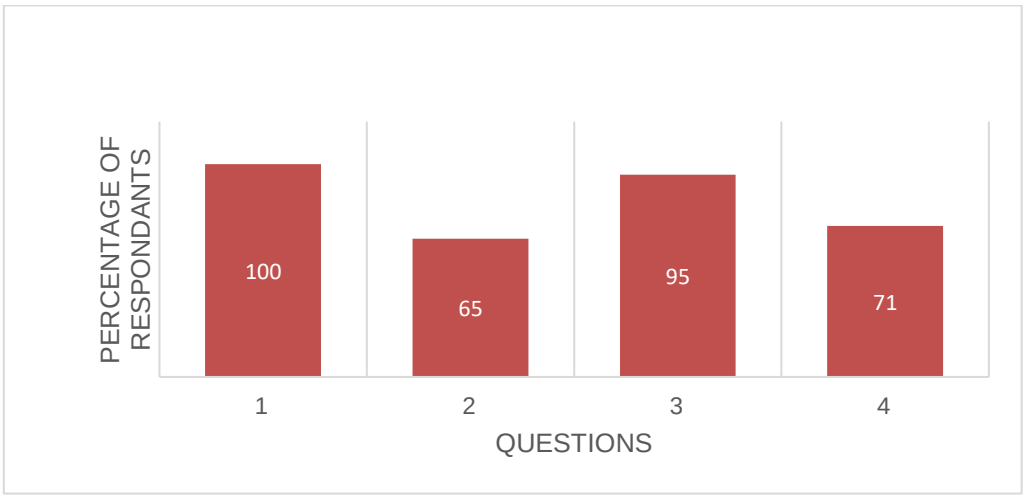


Fig. 29. *Chart demonstrating the results of the survey. Over all the project was well received. All of the respondents thought the study was a good idea and 65% of parents and children were prepared to take some extra time at routine appointments for measurements to be taken. The majority (95%) preferred to have measurements taken at routine appointments and 71% did not want to return another day.*

2.3.2 Experiment 1 results

Part 1

For this experiment 39 patients attending the Eye Department at BCH for outpatient appointments were invited to join the study of which 5 did not fit the inclusion criteria and were therefore not recruited (Fig. 30). The study was well received by the participants and their parents with a total of 34 agreeing to take part giving rise to a recruitment rate of 89% and a completion rate of 100% with all participants completing the measurements.

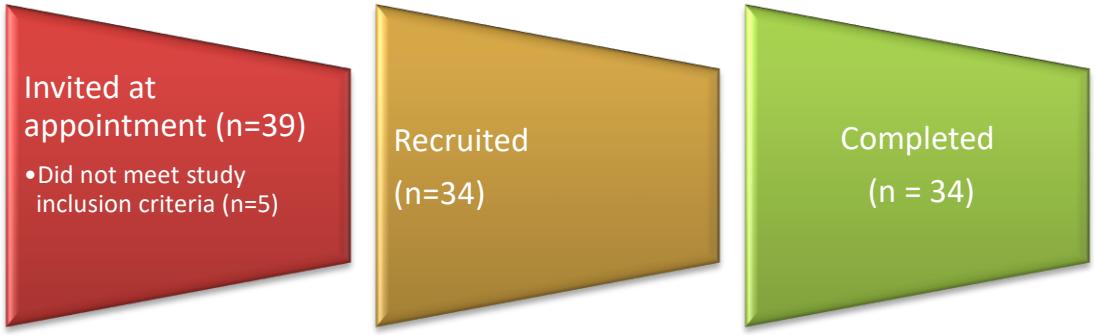


Fig. 30. *This flow diagram demonstrates the recruitment and completion of participants in experiments 1 and 2. The participants that did not fit the inclusion criteria were unwilling to undergo GAT.*

The eyes of 34 children with glaucoma aged 4 to 15 years with an average age of 9 and median age of 8.5 years were included. The majority of the children were male (n = 20) and 14 were female. Most were White (n = 21), 12 were Asian and 1 was Mixed (Gov.UK, 2019). The right eye was chosen in 21 children and the left eye was selected in 13. The children presented with a wide range of refractive error (see table 9c).

	Minimum	Maximum	Mean	SD
GAT	9.0	38.0	17.6	7.1
RBT_{on}	9.0	48.0	20.0	9.1

				Limits of agreement	
	Mean	SD	*p*	Mean – (1.96 x SD)	Mean+(1.96 x SD)
RBT_{on} - GAT	2.4	3.0	0.000	-3.48	8.28

Values are in mmHg

Table 8. *Descriptive statistics for RBT_{on} and GAT (n = 34) and Mean difference, significance level and 95% confidence interval limits between RBT_{on} and GAT.*

	Minimum	Maximum	Mean	SD
GAT	10.0	29.0	16.5	5.9
RBT_{on}	9.0	34.0	17.9	6.9

				Limits of agreement	
	Mean	SD	*p*	Mean – (1.96 x SD)	Mean+(1.96 x SD)
RBT_{on} - GAT	1.4	2.4	0.027	-3.3	6.1

Values are in mmHg

Table 9a. *Descriptive statistics for RBT_{on} and GAT for age group 0 to 9 years (n = 18) and Mean difference, significance level and 95% confidence interval limits between RBT_{on} and GAT.*

	Minimum	Maximum	Mean	SD
GAT	9.0	38.0	22.3	8.3
RBT_{on}	12.0	48.0	18.9	10.8

				Limits of agreement	
	Mean	SD	*p*	Mean – (1.96 x SD)	Mean+(1.96 x SD)
RBT_{on} - GAT	3.4	3.3	0.001	-3.1	9.9

Values are in mmHg

Table 9b. *Descriptive statistics for RBT_{on} and GAT for age group 10 to 15 years (n = 16) and Mean difference, significance level and 95% confidence interval limits between RBT_{on} and GAT.*

	Minimum	Maximum	Mean	SD
Sph	-20.0	+21.0	+4.3	9.9
Cyl	-0.50	-6.00	-1.1	1.5

Values are in dioptres

Table 9c. *Descriptive statistics for refraction data for 34 children with glaucoma. Sph = sphere, Cyl = cylinder (minus form).*

GAT measurements ranged from 9 to 38 mm Hg and RBT_{on} ranged from 9 to 48 mm Hg. The mean IOP ± SD as measured by RBT_{on} was 20.0 (SD 9.1) mmHg versus GAT readings of 17.6 (SD 7.1) mmHg which represents significant positive correlation ($r = .919$, $n = 34$, $p = 0.01$) (Fig. 31) with a significant overestimate of 2.4 (SD 3.0) mmHg ($Z = -3.741$, $p = 0.000$) the bias of which increased significantly with higher IOPs above 28 mmHg (Fig. 31a). The coefficient of determination, $r^2 = 0.84$. The 95% confidence interval of the difference value of (RBT_{on} – GAT) as demonstrated by the Bland-Altman graph was -3.8 to 8.3 mmHg (Fig. 32).

In 62% of the study population, RBT_{on} measurements were within ±3 mmHg of GAT, 41% were within ±2 mmHg and 32% were within ±1 mmHg. Approximately one third (38%) of RBT_{on} measurements were > 3mmHg and 15% were ≥ 5 mmHg than GAT.

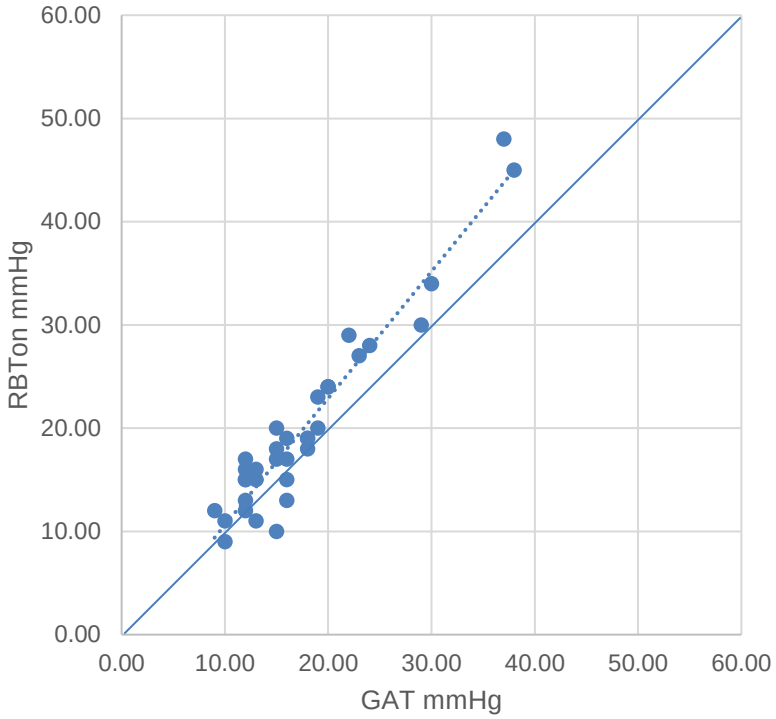


Fig. 31a. *Scatter plot of RBT_{on} versus GAT representing all of the data (y = 1.2281x - 1.665, blue dotted line). The coefficient of determination r^2 = 0.92. This can be expressed as a percentage, demonstrating that 92% of the increase in RBT_{on} in this range was dependent on GAT. However, it can be seen that the majority of the readings were in the range 10 to 20 mmHg. The solid blue line is the line of unity that illustrates the over and underestimation of RBT_{on} when compared with GAT. It demonstrates that approximately 14% of RBT_{on} measurements were lower than GAT, 6% were the same and 80% were higher in the whole cohort.*

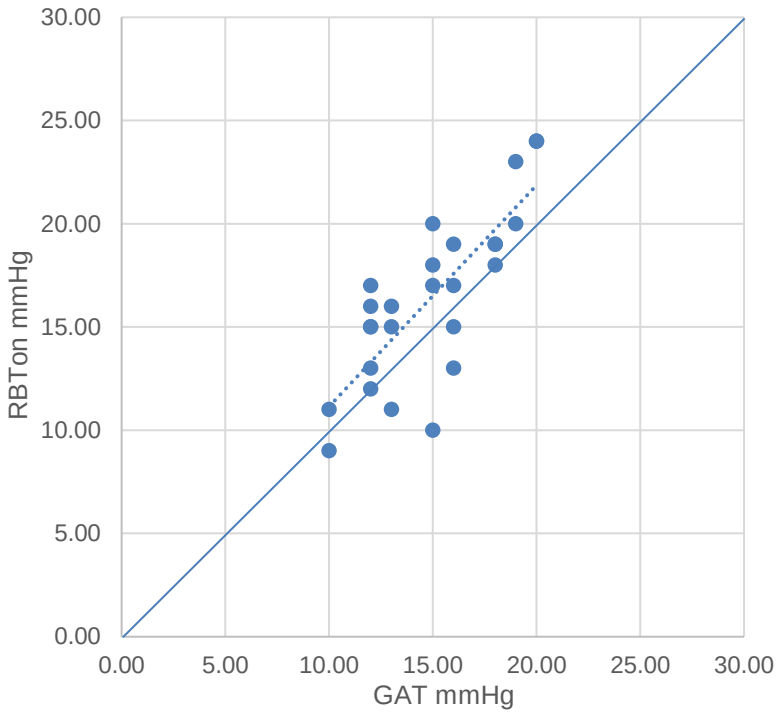


Fig. 31b. *Scatter plot of RBT_{on} versus GAT (y =1.0722x + 0.4259, blue dotted line). Most of the GAT measurements recorded by the present study lay within the clinical range of 10 to 20mmHg. Therefore, this graph represents this clinical range. The coefficient of determination r^2 = 0.63. This can be expressed as a percentage, demonstrating that 63% of the increase in RBT_{on} in this range was dependent on GAT. The solid blue line illustrates the over and underestimation of RBT_{on} when compared with GAT. It demonstrates that 23% of RBT_{on} measurements were lower than GAT, 9% were the same and 68% were higher in this range.*

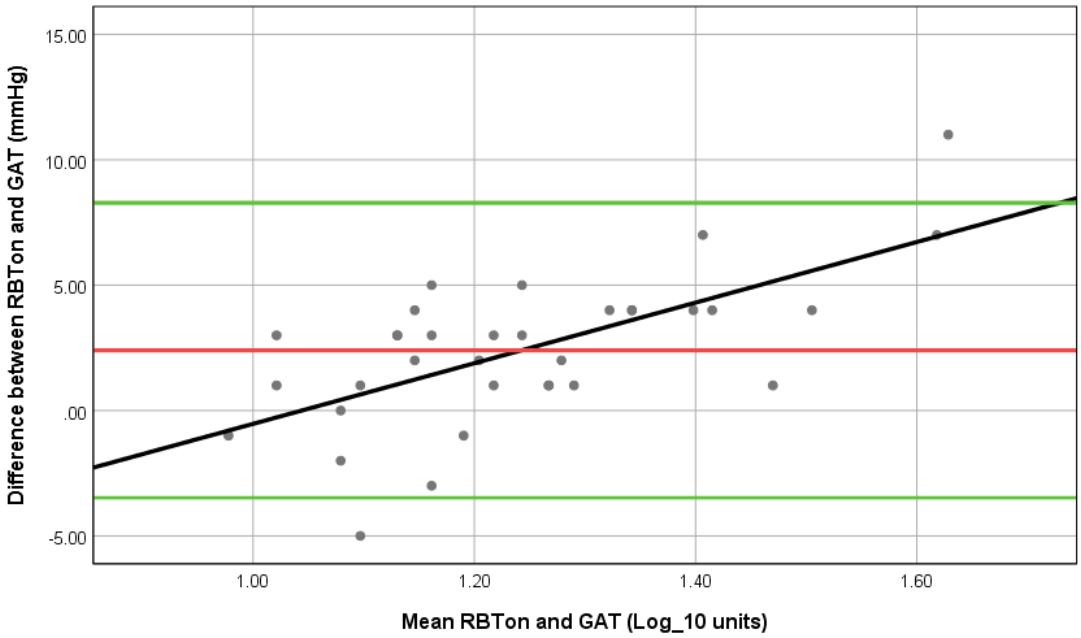


Fig. 32. *Bland-Altman plot of the difference vs the mean, demonstrating the comparison of RBT_{on} and GAT . The red line indicates the mean, the green lines indicate the 95% limits of agreement and the black diagonal line demonstrates the linear regression (y= -12.62 + 12.09*x).*

Part 2

A Fisher's Exact Test of probability indicated a significant association between age group and IOP status A, p = 0.02. The younger age group had more (RBT_{on} - GAT*)* measurements between -5 to 2 mmHg than the older children (Fig. 33).

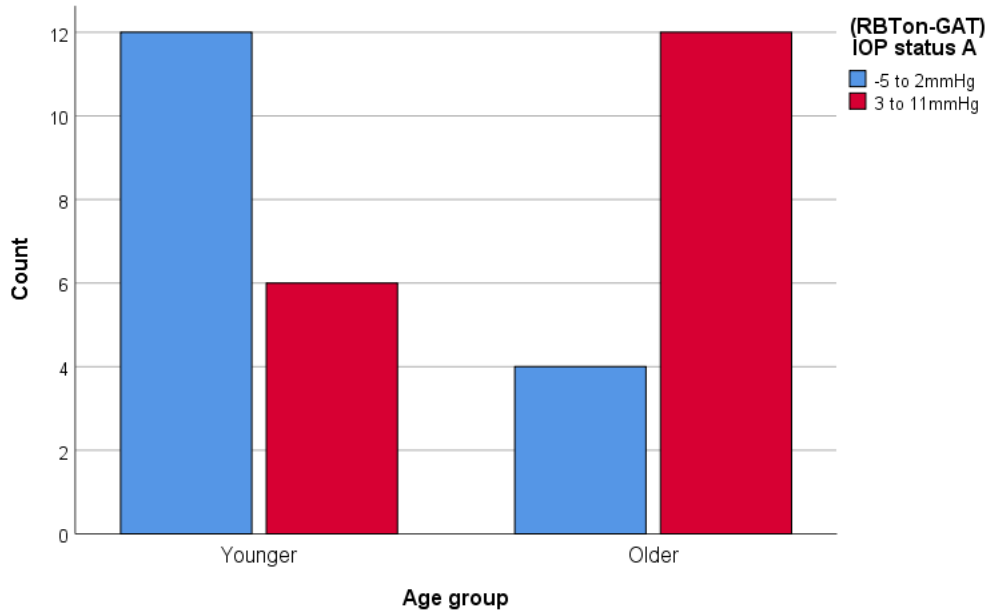


Fig. 33. *The bar chart shows that the range of the difference between RBT_{on} and GAT readings were between -5 and 2 mmHg in the majority of the younger age group (0 – 9 years) and between 3 and 11 mmHg in the majority of the older age group (10 – 15 years). A significant association between the age groups and (RBT_{on} - GAT) was found, p = 0.02. The difference between RBT_{on} and GAT may vary according to the age of the child. However, it should be noted that the older children tended to have higher IOPs where larger differences between RBT and GAT may be expected (Flemmons et al., 2011b).*

RBT_{on} was closer to GAT in children under the age of 10 years (Figs. 9a & 9b). (RBT_{on} - GAT) in the -5 to 2 mmHg group = 0.1, SD 1.8 mmHg. (RBT_{on} - GAT) in the 3 to 11 mmHg group = 4.1, SD 2.6 mmHg. Approximately 17% of RBT_{on} measurements were lower than GAT (mean RBT_{on} – GAT: -2.67 SD 2.1 mmHg) in this age group (Fig. 34), although the difference was < 3 mmHg and therefore, not clinically significant (Dahlamnn-Noor *et al*., 2013). Both GAT and RBT_{on} IOPs increased slightly with age but the increase was not dependent on it (Figs. 35 and 36).

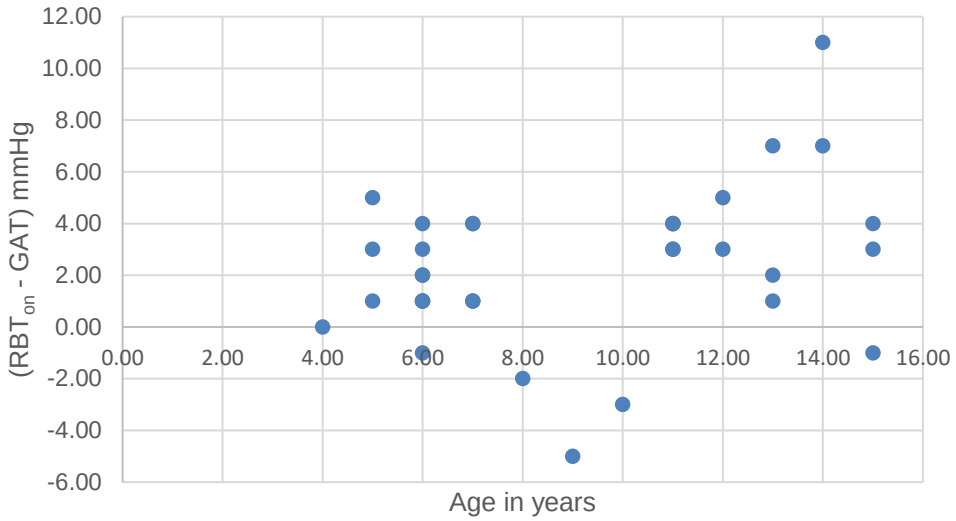


Fig. 34. *This chart shows that RBT_{on} was slightly closer to GAT in children under 10. Approximately 17% of the RBT_{on} measurements in this younger age group were lower than GAT. In children above 10, 90% of RBT_{on} readings were above GAT and were not as close. However, $r^2 = 0.1065$, which indicates that the difference between RBT_{on} and GAT was not dependent on age.*

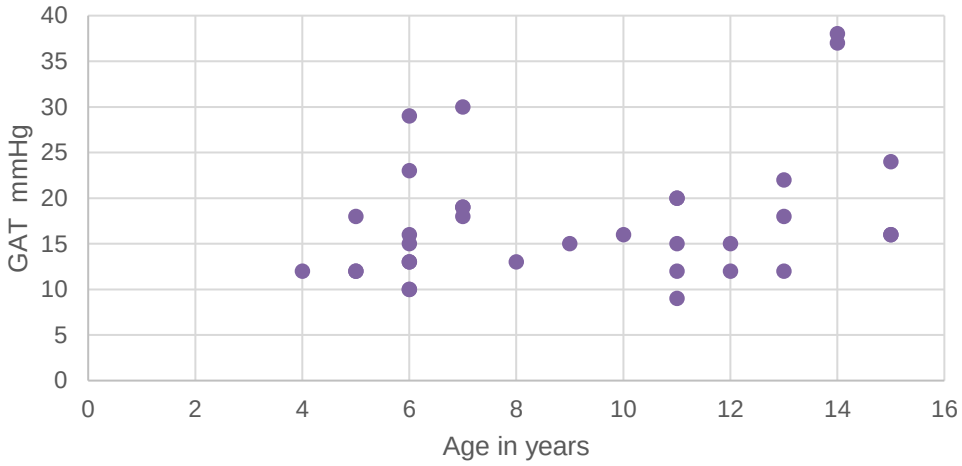


Fig. 35. *Graph of GAT in mmHg versus age of child in years; demonstrating the increase of IOP with age. However, $r^2 = 0.0889$, which indicates that the increase in IOP as measured by GAT was not dependent on age.*

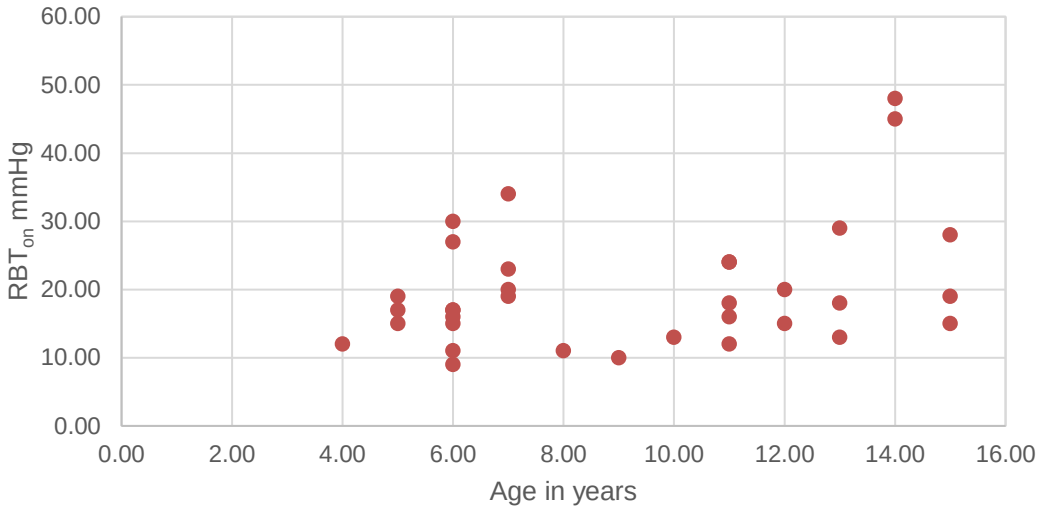


Fig. 36. *Graph of RBT_{on} in mmHg versus age of children with glaucoma in years, demonstrating the general increase of IOP with age. However, $r^2 = 0.1112$ which indicates that an increase in RBT_{on} was not dependent on age.*

A Fisher's Exact Test of probability indicated no significant association between the age groups and IOP status B (group 1. ≤ 3 mmHg and group 2. > 3 mmHg) p = 0.29. (RBT_{on} - GAT) in the ≤ 3 mmHg group = 1.1, SD 1.8 mmHg. (RBT_{on} - GAT) in the > 3 mmHg group = 4.5, SD 3.5 mmHg.

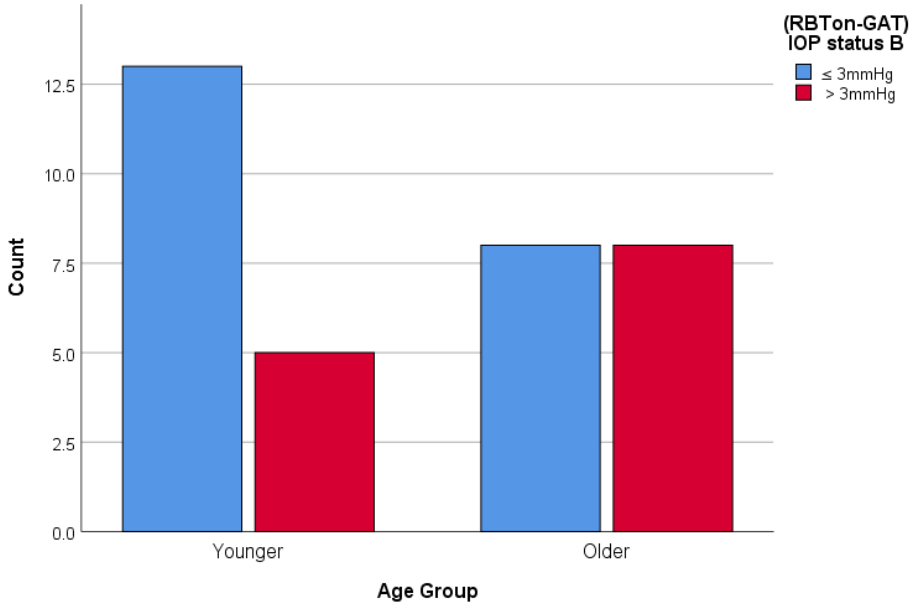


Fig. 37. *Bar chart of (RBT_{on} – GAT) for IOP status B versus age groups demonstrates that the majority of the difference between RBT_{on} and GAT is ≤ 3 mmHg in the younger age group, with the older age group showing no preference. As the difference was not significant it infers that younger children under 9 are not more associated with (RBT_{on} – GAT) measurements that are ≤ 3mmHg or > 3 mmHg than older children.*

2.4 Discussion

2.4.1 Survey

The results of this survey indicated that the study would be well received, although 35% of respondents did say that they would mind the extra time taken. However, this was not a problem as patients spent a great deal of time in the waiting room in between tests and the study was often conducted during this time. Most respondents preferred not to return another day, although 29% indicated that they did not mind. The parents and children were on the whole very positive about joining the study.

2.4.2 Experiment 1

Part 1

The first part of Experiment 1 was designed to look at the validity and reliability of RBT_{on} measurements when compared with GAT which, in spite of its limitations, is the global gold standard of IOP measurement (Gao *et al.*, 2017). Most studies thus far have concentrated on the adult population. Few have compared RBT with GAT in children solely with glaucoma (Dahlmann-Noor *et al*., 2013), with most including children with suspect glaucoma and other conditions (Flemmons *et al.*, 2011b; Grigorian *et al*., 2015). All of the participants in Experiments 1 of the present study had some form of childhood glaucoma. Data for Experiments 1,2, and 3 were obtained from the same cohort. The type of glaucoma was divided into 5 groups (see section 3.3.2).

The main finding from the first part of Experiment 1 of the present study is that RBT_{on} is significantly higher than GAT for IOPs between 8 and 38 mmHg, with this difference increasing at higher IOP's above 28 mmHg (Fig 31a). RBT_{on} overestimated GAT by an average of 2.4 (SD 3.0) mmHg with 95% limits of agreement of -3.8 to 8.28 mmHg, which compares favourably with previous studies in children and adults (Flemmons *et al*., 2011b, Dahlmann-Noor *et al*., 2013; Beasley *et al*., 2013; Poostchi *et al*., 2009; Kim *et al*., 2013; Grigorian *et al*., 2015). However, although this is a statistically significant difference, it is not clinically significant because differences of ± 3 mmHg or less do not generally impact the management of the disease (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013). Davies *et*

al. (2006) referred to larger differences of ± 4 mmHg as being of clinical significance as this was advocated previously by Phelps and Phelps (1976).

As previously mentioned in section 1.8.8.2, an advantage of the RBT device is that it does not need to be recalibrated. Sahin *et al*., (2007) in their study on 152 healthy school children, found similar mean IOP and standard deviation values for examiner's 1 and 2 on right eyes (examiner 1 = 16.48 ± 2.82 mm Hg, examiner 2 = 17.27 ± 3.27 mm Hg). They also found good intra-observer coefficients for repeated readings for both examiners (examiner 1 = 0.970, examiner 2 = 0.963, $P<0.0001$), with 1.0 being perfection. This demonstrates that normal variability is low with the RBT device, although it was not possible to measure intra-observer coefficients in the present study due to the clinical setting.

Frequently used Goldmann tonometers in clinical settings are particularly subject to calibration errors that can only be fixed by the manufacturer. Typically, manufacturers tolerance for errors is usually ±0.5 mmHg at all levels of testing*,* but it is possible to check calibration errors in clinical settings (Sandhu *et al*., 2005). Choudhari *et al*., (2009) tested 132 Haag-Streit Goldmann tonometers (Haag-Streit, Bern, Switzerland) of the same model and found only 4% were within ±0.5 mmHg at 20 mmHg and 28% were within ±2.0 mmHg. At IOPs of 0 and 60 mmHg 12.12% were more than ±2.0 mmHg different. The South East Asia Glaucoma Interest Group issued guidance regarding acceptable calibration error at the following testing levels: 0mmHg ±2.0 mmHg, 20mmHg ±3.0 and 60 mmHg ±3.0 mmHg (Choudhari et al., (2009). Sandhu *et al*., (2005) also recommend that errors of ±2.5 mmHg at all IOPs are acceptable clinically. This supports the conclusion of the present study, that although a statistically significant mean difference of 2.4 ±3.0 mmHg between RBT_{on} and GAT was found, it was not clinically significant because the difference is less than the acceptable calibration error of GAT.

Similar studies on children have found a significant difference between RBT and GAT (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013) as have some adult studies (Abraham *et al*., 2008; Poostchi *et al*., 2009; Beasley *et al.*, 2013). However, Davies *et al*. (2006) did not find a significant difference between RBT and GAT, possibly because their study was undertaken on optometry students who would have cooperated well.

Study	Year	Age range in years	% RBT-GAT ≤ 3 mmHg	Mean difference/SD RBT- GAT (mmHg)
Martinez de la Casa *et al.*	2009	3 to 13	N/A	+3.10 (SD 4.0) (HAT)*
Flemmons *et al.(b)*	2011	4 to 17	63	+2.30 (SD 3.7)
Dahlmann-Noor *et al*.	2013	4 to 19	63	+ 3.30
Grigorian *et al*.	2015	7 to 17	N/A	+1.53 (SD 3.75)
Borrego Sanz *et al*.	2016	0 to 7	N/A	+0.42 (SD 3.69) (HAT)*

* HAT = Hand held applanation tonometer (Perkins)

Table 10. *Values obtained from previous paediatric studies*

RBT_{on} measurements were within ±3 mmHg of GAT in 62% of participants, which compares favourably with previous studies by Flemmons *et al*. (2011b), Dahlmann- Noor *et al*., (2013) and Grigorian *et al*. (2015) (Table 10). Other studies of the adult population with and without glaucoma and have higher levels of agreement within ±3 mmHg (Table 11). This may be due to adults cooperating better during measurement, with less lid squeezing and movement. Approximately one third of RBT_{on} measurements differed from GAT by > 3 mmHg and 15% differed by > 5 mmHg which is clinically significant. Therefore, the present study agrees with Flemmons *et al*. (2011b) and Dahlmann-Noor *et al*. (2013), that RBT is useful as a screening tool and that in severe cases of glaucoma where small IOP alterations are critical, assessment by a second tonometer is advisable e.g. in bilateral normal tension glaucoma a 1 mmHg difference between two eyes can result in more field loss in the eye with the higher IOP (Crichton *et al*., 1989).

Study	Year	Age range in years	% RBT-GAT ≤3 mmHg	Mean differences/SD RBT and GAT mmHg	Number of participants
Fernandes *et al.**	2005	17 to 30	80.0	+1.43 (SD 2.03)	46
Beasley *et al*.*	2013	> 18	N/A	+2.7 (SD 2.8)	36
Iliev *et al.***	2016	> 18	84.1	+1.0 (SD 2.17)	55
Gao *et al.***	2017	23 to 81	78.3	-0.22 (SD 3.07)	336

Table 11. *Values obtained from previous adult studies. Presence of glaucoma: * no, ** mixed.*

A significant positive correlation (r = 0.96, p = 0.01) was found between RBT_{on} and GAT which is similar to several previous paediatric and adult studies (Davies *et al*., 2006; Johannesson *et al*., 2008; Poostchi *et al*., 2009; Gao *et al*.,2017). However, Iliev et al., (2006) found a weak correlation between RBT and GAT in their adult study. The coefficient of determination, r^2 = 0.92, demonstrates how close the data fit to the regression line and can be expressed as a percentage, therefore 92% of the variance is accounted for by the regression line.

A high degree of consistency was found between RBT_{on} and GAT. Figure 31a represents the relationship between RBT_{on} and GAT for the whole cohort but also demonstrates that the majority of GAT readings were between10 to 20mmHg. Figure 31b represents the relationship between RBT_{on} and GAT for the IOP range from 10 to 20mmHg. As these figures show, r^2 = 0.92 for the whole cohort and for the 10 to 20 mmHg cohort r^2 = 0.64. By eliminating the few lower and higher readings, it is possible to get a better understanding of the relationship between RBT_{on} and GAT for this smaller range of IOP. In this smaller range only 64% of RBT_{on} can be explained by GAT. Therefore, other factors such as variation in RBT probe position, patient cooperation etc. may influence the relationship between these two instruments. The lines of agreement in Figs 31a and 31b illustrate the over and under estimation of RBT_{on} compared with GAT. For the whole cohort, 14% of RBT_{on} underestimated GAT, 6% was the same and 80% overestimated. It was also found that in the smaller normal range group 23% of RBT_{on} underestimated GAT, 9% were the same and 68% overestimated. Chui *et al*., (2008) and Yamashita *et al*., (2011) found that RBT taken at the centre of the cornea tended to be lower than GAT at lower IOPs. However, they did not state what these lower IOPs were. Muttuvellu *et al*., 2012 also found that central RBT underestimated GAT for IOPs < 11.6 mmHg and overestimated at higher. The result from the present study was interesting because it found that RBT underestimated GAT the majority of the time in IOPs < 10 mmHg. The smaller cohort of 10 to 20 mmHg is more representative of a normal IOP range (see section 1.4). The higher IOPs above 24mmHg recorded by the present study were generally caused by poorly controlled glaucoma which required urgent review.

The present study had a relatively small but powered sample size (n= 34) due to the difficulty in recruiting children with glaucoma within a reasonable length of time. Paediatric glaucoma is a rare disease. Therefore, this study was undertaken in a Hospital Eye Department with

specialized clinics for children with glaucoma. Children and their parents often travelled long distances to attend these clinics. Most previous studies that compare RBT with GAT in paediatric populations also have small sample sizes, i.e. Poostchi *et al*., 2009 (n=100), Flemmons *et al*., 2011b (n= 71), Dahlmann-Noor *et al*., 2013 (n = 74). Grigorian *et al*., 2015 undertook a larger study (n = 214). However, their cohort included children with glaucoma, suspect glaucoma and other eye diseases. Further larger studies are needed to look at the clinical application of RBT in children with glaucoma.

The present study was undertaken in a single centre clinical setting and was subject to certain limitations as a result. Most of the measurements were unmasked. Only one measurement was taken, often by the same observer, as it was not possible to conduct this any other way in this very busy clinic, which may have introduced some bias. Other clinic-based studies have also collected data unmasked and by more than one observer (Iliev *et al*., 2006, Lundvall *et al*., 2011). The order of measurement was not randomized with RBT being taken first before a topical anaesthetic was administered for GAT because GAT can cause displacement of fluid during measurement. This method was also used by several other studies (Fernandes *et al*., 2005, Iliev *et al*., 2006, Flemmons *et al*., 2011b, Dahlmann-Noor *et al*., 2013). It is possible that children found RBT stressful as it was the first measurement and some may have held their breath in anticipation leading to higher IOPs than GAT. Vera et al., (2019) noted that modified breathing can cause an increase of IOP up to 8 mmHg. The same could also be said for GAT due to its invasive nature. However, all of the children were accustomed to having their IOPs measured by both GAT and RBT so, this is unlikely in the present study.

The children who chose to participate in the present study were used to visiting the Eye Department, and cooperated well. They often had to wait a long time in the waiting room in between tests and were happy to pass the time by taking part in this study. Indeed, some of them had taken part in previous studies at BWCH.

Approximately 90 % of measurements were undertaken by Nicola Sabokbar and 10 % were taken by Mr Joseph Abbott, both of whom are highly experienced at using RBT and GAT. Mr Abbott is a consultant ophthalmologist with a special interest in paediatric glaucoma and Nicola Sabokbar is a specialist independent prescribing optometrist with many years of

experience of using GAT and RBT. This was important regarding GAT measurements which were technically more difficult to perform than RBT. A study by Abraham *et al*. (2008) found that RBT compared favourably with GAT when taken by both experienced and inexperienced technicians however this was not true for GAT where experience was necessary.

Inter-rater agreement is important as it reflects the cohesion of variables being measured by different investigators, however, perfect agreement is not usually achieved (McHugh, 2012). As this study was undertaken in a clinical setting, it was not possible to quantify the inter-rater agreement between Mr Joseph Abbott and Nicola Sabokbar. However, to reduce the error that might be introduced, as part of preparation for the study, Mr Abbott observed and checked a GAT measurement made by Nicola Sabokbar and was satisfied with the result. In the end, the majority of measurements were taken by Nicola Sabokbar, so it is unlikely that the different operators had much influence on the results.

Another limitation of this study was that the participants had a wide range of paediatric glaucoma with different treatments which may have affected IOP measurement due to changes in CCT and corneal biomechanics (Agarwal *et al*., 2012). In addition, diurnal variation, which can vary by 6 mmHg per day in children with glaucoma (Tajunisah *et al*., 2007), could not be taken into consideration (see section 1.4). It was not possible to obtain all of the readings at a certain time of day because clinics were held both in the morning and afternoon. Davies *et al*. (2006) ensured that all of their measurements were taken at the same time of day. However, their study was not conducted in a clinical setting.

Part 2

The second part of Experiment 1 examined the association between age and the difference between RBT_{on} and GAT for IOP status A and status B. A significant association between the child's age group and IOP status A was found. No association between age group and IOP status B was found, which agrees with Flemmons *et al*. (2011b) who divided their IOP the same way.

Tables 9a and 9b demonstrate that RBT_{on} was significantly different to GAT in both age groups and that the mean difference between RBT_{on} and GAT was lower in the younger age group (0-9 years). The older age group (10-15 years) had a larger mean difference, wider

limits of agreement and higher standard deviations. However, it should be noted that the older group also had a wider range with higher readings. Dahlmann-Noor *et al*., 2013 and Flemmons *et al*., 2011b found differences between RBT and GAT increased at higher IOPs above the “normal” range in children with glaucoma which might explain the larger mean difference in the older age group in the present study. They do not state what they mean by normal, however, their graphs indicate that the larger mean difference occurred above 20 mmHg**.** Similarly, Poostchi *et al*., (2009) in their study of 100 participants aged 9 to 84 years with and without glaucoma found that the difference between mean RBT and GAT was significantly greater at higher IOPs. Again, higher IOPs are not defined, however, further examination of their paper suggests that this would be IOPs above 20mmHg. Munkwitz *et al*., (2008) confirm this finding. They examined 75 adults with a range of ocular disease and found that the difference between RBT and GAT was twice as large in the IOP range of 23 to 60 mmHg than in the 7 to 22 mmHg range.

The higher standard deviations, indicating more variability in the older age group, were unexpected as it was expected that this age group would be more experienced at having their IOPs measured. However, the younger age group were very experienced at having their IOPs measured and the author noted their positive attitude and good cooperation.

The higher mean difference of 3.4 mmHg in the older age group is clinically significant, as most previous paediatric studies agree that differences > 3mmHg are clinically important as they may impact the management of glaucoma. (Dahlmann-Noor *et al*., 2013; Flemmons *et al*., 2011b).

In the present study 1/3 of RBT_{on} was lower than GAT in children 9 years and under. As GAT has been found to be lower than the real IOP as measured by manometry (Kniestedt *et al*., 2008), RBT in young children may be even further from the true IOP. The present study found that 90% of RBT readings in children over 9 years were higher than GAT which is similar to findings in previous studies in adults (Beasley *et al*., 2013; Davies *et al*., 2006, Fernandes *et al*., 2005). Therefore, the present study recommends that practitioners should be aware when using GAT in children that may only compare well with adults from the age of 10 or 11 when corneal thickness reaches adult levels (Eisenberg *et al*., 1998). The present study also

recommends that RBT should not be used as a substitute for GAT in children aged 9 years and under, in critical cases. A second tonometer should be used to verify RBT measurements. Eisenburg *et al*., (1998) found that mean IOP by applanation tonometry is lower in children than adults reaching adult levels by 10 years of age and suggest the following equation can be used to calculate the normal IOP of a child below 10 years of age; IOP (applanation) = (0.71 x age + 10) mmHg. They also found that GAT and the NCT had a linear relationship up to the age of 10 years beyond which the linear fit deteriorated. No association of age with NCT and age with TonPen was found. Similarly, the present study found that IOP in children with treated glaucoma as measured by RBT and GAT, rises until the age of 7 years, following which it decreases and starts to rise again from the age of 10 (Figs. 35 and 36). Eisenburg *et al*., (1998) also found that GAT is affected by CCT, with an increase in CCT of 100 μm producing an increase in IOP of 2.739 mmHg. Doughty and Zaman (2000) agree with Eisenburg *et al*., (1998) but found that a 100 μm CCT increase produced a slightly smaller change of 2.2 mmHg. Jaafar and Kazi (1993) found that IOP is lower in young children under 5 years than in adults when measured by the hand held Perkins applanation tonometer in the supine position and becomes equal around 12 years old. However, no difference was found with NCT. Hashemi *et al*., (2018) used the NCT to examine the normal range of IOP in a young adult population (age 20 – 34 years) in Iran and found a statistically significant increase of IOP with age. Age has been found to have an association with an increase in IOP in adult populations in the Barbados Eye study, The Beaver Dam Study and the Egna-Neumarkt Study (Klein *et al*., 1992; Leske *et al*.,1997; Bonomi *et al*., 1998). However, Nomura *et al*. (2004), found a decrease in IOP with age in the adult Japanese population.

2.5. Conclusion

RBT correlates well with GAT in children with glaucoma (r = .919, n=34, p=0.01) within the typically encountered range of 10 to 28 mmHg. Mean RBT overestimated IOP by 2.4 (SD 3.0) mmHg when compared with GAT, however this difference is less 3 mmHg so is not clinically important. One third of RBT readings in children with glaucoma differ from GAT by over 3 mmHg, so RBT needs back up from another instrument for some cases where small IOP changes are important. By contrast in adults only 20% of RBT readings differ from GAT by

more than 3 mmHg. The present study recommends that further randomized trials are needed to compare RBT with GAT in young children with glaucoma.

A significant association between the child's age and IOP status A was found between RBT_{on} and GAT, with RBT_{on} behaving differently in relation to GAT in younger children. The majority of RBT_{on} readings in children above 10 were higher than GAT, which agrees with previous studies in adults. In addition, IOP in children with treated glaucoma was found to increase until the age of 7 years. The difference between RBT_{on} and GAT versus IOP status B, was not associated with the child's age. The above considerations should be taken into account when measuring IOPs in children 9 and under with RBT and GAT. Therefore, the present study recommends that a second tonometer should be used to verify measurements in critical cases

Chapter 3

The reliability of suboptimal Rebound tonometry measurements in children with glaucoma.

3.1 Introduction

RBT measurements should ideally be carried out at the centre of the cornea. However, this is not always possible in young children. The second experiment in this study was designed to establish the reliability of RBT_{off} measurements when compared with RBT_{on}. Research on suboptimal RBT measurement thus far has concentrated on adult populations (Queirós *et al*., 2007, Yamashita *et al*., 2011, Muttuvellu *et al*., 2012, Beaseley *et al*., 2013). Previous studies have mostly concentrated on healthy eyes. Indeed, as far as the author is aware, none have been found to include children either with or without glaucoma. Clinicians need to know how reliable these readings are when treating children with glaucoma (Joseph Abbott, personal communication, 2016). GAT is affected by corneal thickness, disease and surgery which can change ocular rigidity leading to over or under estimation of IOP (Damji *et al*., 2003). It is important to examine the validity of RBT_{off} measurements in young or uncooperative children and those with corneal scars or disease, as GAT may be unreliable or impossible and these may be the only values available without subjecting the child to a general anaesthetic (Yamashita *et al*., 2011; Dahlmann-Noor *et al*., 2013). Previous studies in adults have indicated that temporal RBT measurements are close in value to central and GAT measurements. Therefore, the temporal position is possibly good alternative where central measurement is not possible (Chui *et al*., 2008, Yamashita *et al*., 2011). It would be useful to see if this is was similar in children with glaucoma.

As previously mentioned in 1.5.2, children with glaucoma are more likely to have co-existing conditions e.g. strabismus, nystagmus etc. than their healthy counterparts. As the author is aware nothing is known about the reliability of suboptimal RBT measurements with in children with glaucoma and these co-existing conditions. Therefore Experiment 3 of the present study addresses this.

In order to address these questions, this chapter consists of Experiments 2 and 3. Experiment 2 will examine how temporal suboptimal RBT_{off} measurements compare with central readings

(RBT_{on}) in children with glaucoma and experiment 3 will examine the association of other factors on the mean differences between the RBT_{off} and RBT_{on} measurements.

3.2 Experiment 2: The reliability of suboptimal RBT measurements.

3.2.1 Methods

Experiment 2 shares the same cohort as Experiment 1. RBT measurements were taken at the monocular PD (RBT_{on}) and 3 mm temporally (RBT_{off}) as described previously in section 2.2.2. The eye and position were selected by a pseudorandomized method as described previously in section 2.2.2.

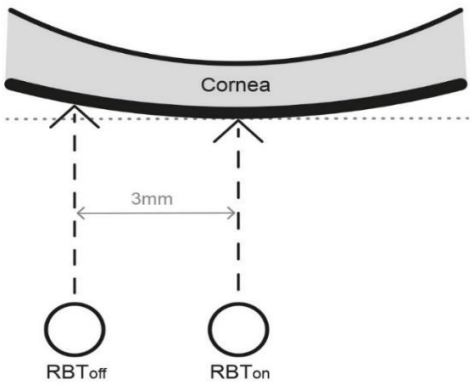


Fig. 38. *A schematic diagram of the probe positioning in relation to the cornea. In addition to the central corneal (RBT_{on}) measurement, a parallel measurement was taken 3 mm temporally (RBT_{off}).*

3.2.2 Statistical analysis

The number of participants was calculated as previously stated in Experiment 1, resulting in a sample size of 34. Data were tested for normality as previously described in section 2.2.3 and transformed into Log_10 Units where necessary.

The non-parametric Wilcoxon Signed Ranks Test was used to indicate whether there was a useful level of agreement between the medians of these measurements because mean RBT_{off} and mean RBT_{on} values did not have normal distributions.

Individual differences between RBT_{off} and RBT_{on} were calculated by subtracting RBT_{on} readings from RBT_{off}. Limits of agreement between transformed RBT_{off} and RBT_{on} data (expressed at 95% confidence level, where the mean of the difference +/- 1.96 SD of the differences) were then demonstrated using a Bland Altman scatter plot. This was followed by

a linear regression procedure to check for linear trend and proportional bias. In addition, the relationship between RBT_{off} and GAT was examined by subtracting GAT from RBT_{off} and following the same procedure as above.

3.2.3 Results

Thirty-four eyes from 34 children with glaucoma were included. A KS test indicated that mean RBT_{off} and mean RBT_{on} were not normally distributed due to some positive skew and a few outliers. RBT_{off} measurements ranged from 9 to 52 mmHg and RBT_{on} ranged from 9 to 48 mmHg. The mean IOP ± SD as measured by RBT_{off} was 19.3 (SD 9.4) mmHg versus RBT_{on} readings of 20.0 (SD 9.1) mmHg which represents a significant positive correlation (r = 0.933, n = 34 p = 0.01) with an insignificant underestimate of -0.71 (SD 2.7) mmHg (Z = -1.647, p = 0.10) the bias of which only increased slightly with higher IOP's above 35 mmHg (Fig. 39a). The coefficient of determination, r^2 = 0.92 and the 95% confidence interval of (RBT_{off} – RBT_{on}) as demonstrated by the Bland-Altman graph was – 6.00 to 4.51 mmHg.

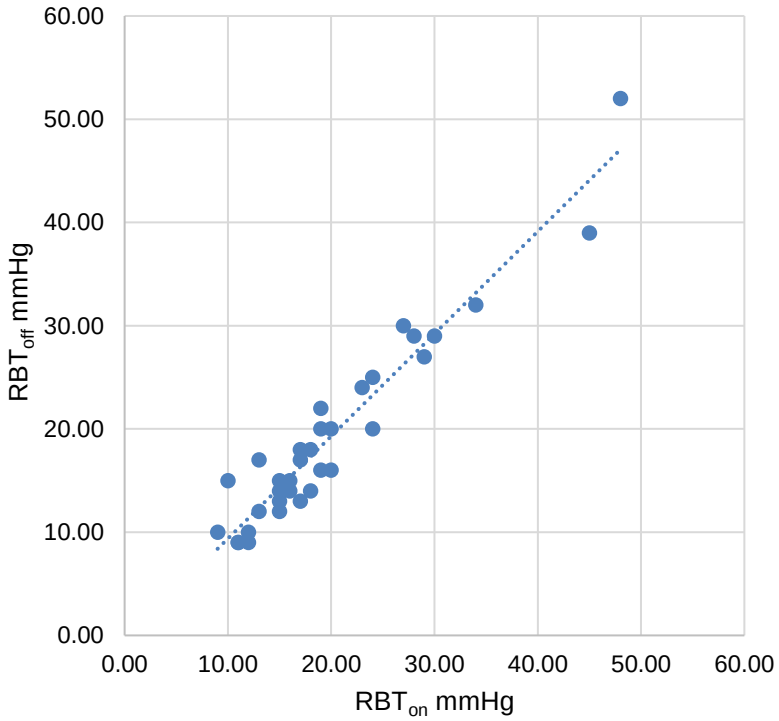


Fig. 39a. *Scatter plot of RBT_{off} versus RBT_{on} mmHg for the whole cohort (y = 0.9916x - 0.5391, blue dotted line). This included the high IOP values. R^2 linear = 0.923. Therefore, 92% of RBT_{off} can be explained by RBT_{on}. The solid blue line of unity illustrates the under and over estimation of RBT_{off} compared with RBT_{on}. RBT_{off} measurements were lower than RBT_{on} in 53% of measurements 12% were equal and 35% were higher.*

	Minimum	Maximum	Mean	SD
RBToff	9.0	52.0	19.3	9.4
RBTon	9.0	48.0	20.0	9.1
GAT	9.0	38.0	17.6	7.1

Values are in mmHg

Table 12. *Descriptive statistics for $RBT_{off,,}$ RBT_{on} and GAT (n = 34)*

				Limits of agreement	
	Mean	SD	*p*	Mean – (1.9 x SD)	Mean + (1.9 x SD)
RBT_{on} - RBT_{off}	-0.7	2.7	0.100	-6.00	4.51

Values are in mmHg

Table 13. *Mean difference, significance level and 95% confidence interval limits between RBT_{off} and RBT_{on}.*

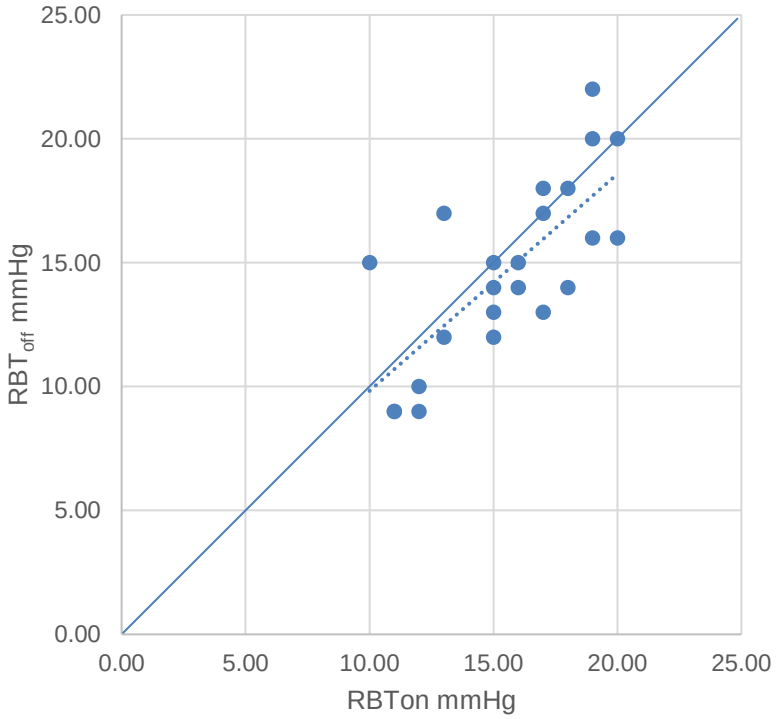


Fig. 39b. *Scatter plot of RBT_{off} versus RBT_{on} mmHg for the data between approximately 10 to 20 mmHg, which represents the majority of IOP values obtained in this cohort (y = 0.8751x + 1.0753 blue dotted line). There were a few higher values, which were removed. R^2 linear = 0.548. Therefore 55% of RBT_{off} can be explained by RBT_{on} in this IOP range. The solid blue line of unity illustrates the under and over estimation of RBT_{off} compared with RBT_{on} in this cohort. RBT_{off} measurements were lower than RBT_{on} in 59% of measurements, 18% were equal and 23% were higher.*

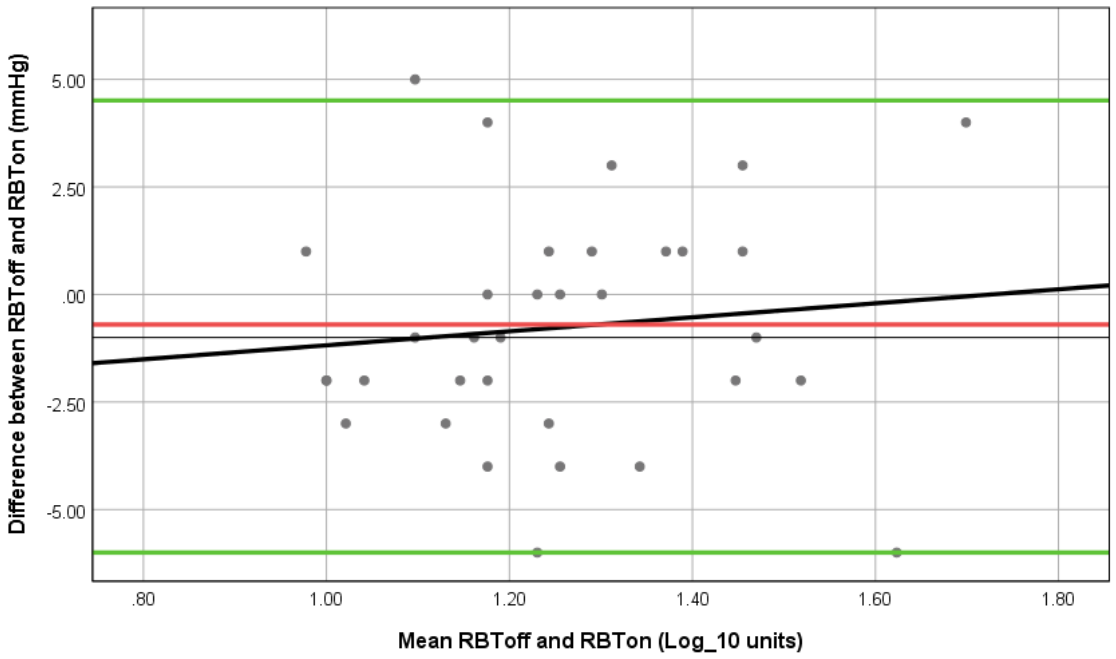


Fig. 40. *Bland-Altman plot of the difference vrs the mean, demonstrating the comparison of RBT_{off} and RBT_{on}. The red line indicates the mean, the green lines indicate the 95% limits of agreement and the black diagonal line demonstrates the linear regression (y = -2.81 + 1.63*x).*

The difference between RBT_{off} and RBT_{on} was within ±1 mmHg in 41.2% of measurements.

The difference between RBT_{off} and RBT_{on} was < 3 mmHg in 62% of measurements and was ≥ 3 mmHg in 38%.

As the mean IOP ± SD as measured by RBT_{off} was 19.3 (SD 9.4) mmHg and the mean IOP ± SD as measured by GAT (see 2.3.2) was 17.6 (SD 7.1) mmHg, it was found that mean RBT_{off} significantly over-estimated GAT by an average of 1.64 (SD 3.5) mmHg with 95% limits of agreement of -5.21 to 8.51mmHg (Z = -2.679, p = 0.007).

RBT_{off} and GAT showed significant positive correlation (r = 0.96, n = 34 p = .01) the bias of which increased slightly with higher IOP's above 30 mmHg. The coefficient of determination r^2 = 0.92.

				Limits of agreement	
	Mean	SD	*p*	Mean – (1.96 x SD)	Mean+(1.96 x SD)
RBT_{off} - GAT	1.64	3.5	0.000	-5.21	8.51

Values are in mmHg

Table 14. *Mean difference, significance level and 95% confidence interval limits between RBT_{off} and GAT. It can be seen that mean RBT_{off} significantly over-estimated GAT.*

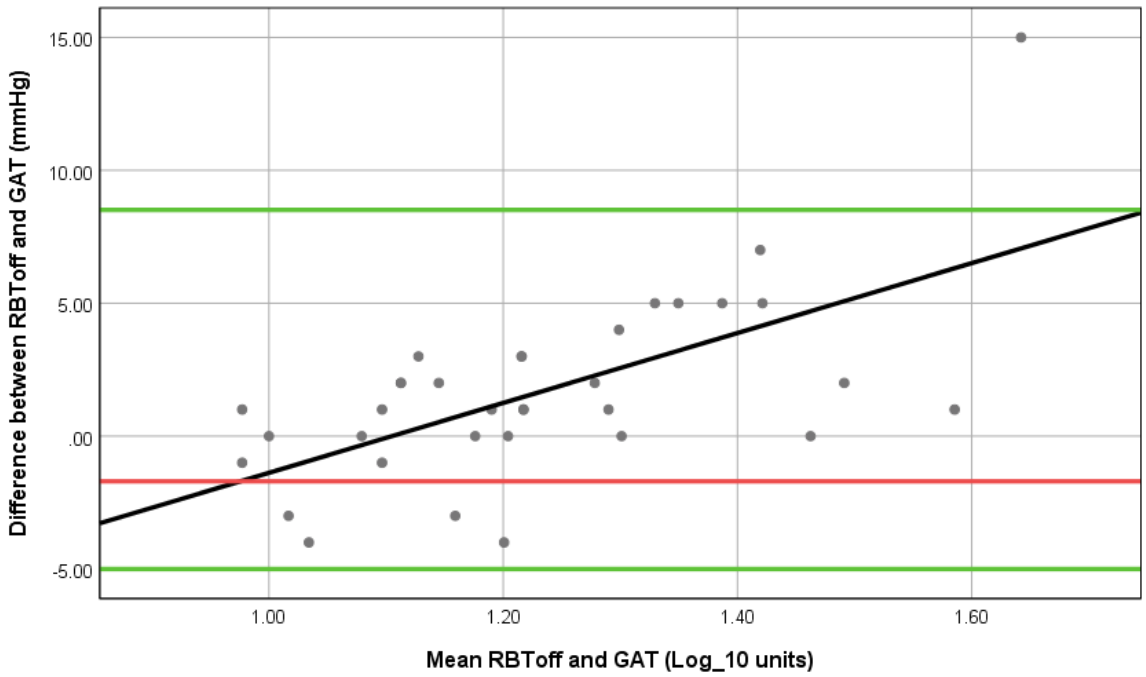


Fig. 41. *Bland-Altman plot of the difference versus the mean, demonstrating the comparison of RBT_{off} GAT. The red line indicates the mean, the green lines indicate the 95% limits of agreement and the black diagonal line demonstrates the linear regression (y = -14.53 + 13.15*x).*

3.2.4 Discussion

The main finding from Experiment 2 is that temporal RBT_{off} is not significantly different from central RBT_{on}. Good agreement was found between RBT_{off} and RBT_{on} from 9 to 48 mmHg (Fig.39a) for the whole cohort (r^2 = 0.923), which is similar to previous studies (Sullivan-Mee and Pham., 2004; Queirós *et al*., 2007). However, a limitation of this study was that the sample size, although powered at 80%, was small with very few high values so this warrants further investigation. A lower level of agreement was found when RBT_{on} measurements above 20mmHg were eliminated (r^2 = 0.548) (Fig.39b). The higher and lower measurements were eliminated because most of the readings were within the more normal 10 to 20 mmHg range (see section 1.4). Relatively few RBT_{on} were above 20mmHg or below 10 mmHg. Therefore, it was possible that the higher and lower readings introduced an unwanted bias. This analysis demonstrates that for IOPs between 10 to 20 mmHg 55% of RBT_{off} measurements were explained by RBT_{on} and 45% were not. Therefore, other reasons may be attributed to the increase of RBT_{off} as RBT_{on} increases such as lack of cooperation by the participants and probe position and angle inaccuracies. The line of unity in Figures 39a and 39b demonstrate the over and underestimation of RBT_{off} compared with RBT_{on} for the whole cohort and for the

RBT_{on} range of 10 to 20 mmHg. RBT_{off} was lower than RBT_{on} in 53% of the whole cohort, was equal in 12% and higher in 35%. By contrast in the smaller 10 to 20 mmHg group RBT_{off} was less than RBT_{on} in 59% of participants, equal in 18% and higher in 23%. As this represents the normal range of IOP without the high and low outliers, the results from this smaller cohort are probably a better representation of the relationship between RBT_{off} and RBT_{on}. Several previous studies have reported that peripheral RBT readings tend to be lower that central and this is discussed further on in this section. As far as the author is aware there are no reports concerning the percentage of over and under estimation of peripheral and central RBT measurements.

Mean RBT_{off} underestimated RBT_{on} by an average of -1.70 (SD 3.5) mmHg with 95% limits of agreement of -8.56 to 5.16 mmHg. This agrees with some previous studies where RBT in the temporal periphery of the cornea has been found to be lower than RBT at the centre (Queirós *et al*., 2007, Muttuvelu *et al*., 2012 and Beasley *et al*., 2013,). However previous results have varied according to the way in which the RBT device has been used (either fixed to a slit lamp or freely held). A smaller insignificant difference of 0.37 mmHg was found by Queirós et al., (2007) with a freely held RBT and a larger significant difference of 3 – 4 mmHg with a fixed device was found by Muttuvelu *et al.,* 2012. Beasley *et al.,* (2013) did not find a significant difference for any peripheral readings and the 5° tilt from central RBT. However, a significant difference was found when the probe was tilted by 10 ° nasally although this was < 1 mmHg so not clinically significant. Yamshita *et al*., (2011) examined nasal, temporal, superior and inferior peripheral corneal measurements and found that nasal and superior values were significantly higher than central RBT, inferior and temporal RBT measurements were also higher that GAT. However, the difference was not significant. Similar studies with the Ton-Pen have had varying results: Mok *et al*., (1999) found that peripheral corneal measurements were significantly higher than the centre; however, Sullivan-Mee and Pham (2004) found that peripheral values were slightly lower but not significantly different. Whiteacre and Stein (1993) found a non-significant difference for peripheral GAT measurements when compared with central.

The Ocular Hypertension Treatment Study (see section 1.6.3) found that GAT measurements were higher in thicker corneas (Brandt *et al*., 2001). Therefore, it was expected that IOP would

be higher in the peripheral cornea due to the 25% increase in thickness (Martola and Baum., 1968) however, the reverse was found. Previous studies have looked at the effect of CCT on RBT with varying results. Martinez-de-la-Casa *et al*., (2005) found a strong correlation between RBT and CCT. However, Queirós *et al*., (2007), Chui *et al*., (2008) and Takenaka *et al*., (2011) did not. As mentioned previously several studies have found that peripheral RBT measurements were lower than central. Takenaka *et al*., (2012) argue that this may be due to the angle of the RBT probe when it hits the peripheral cornea from a straight position due to the curvature of the cornea, reducing the "bounce speed" of the probe. The deceleration of the probe is used to calculate the IOP and a reduction in deceleration will lead to a lower IOP being registered by the device.

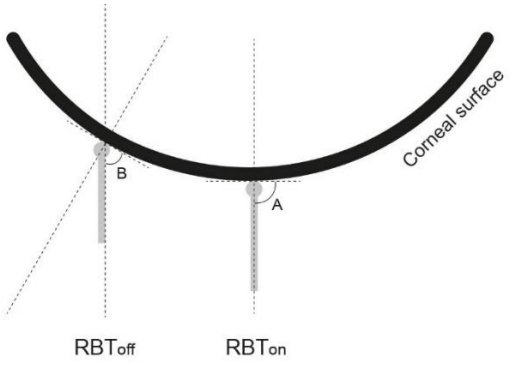


Fig. 42. *This diagram of the probe alignment demonstrates the angle of corneal touch by the probe at the centre of the cornea (A) and the angle by the temporal probe (B). The force of the probe is greatest when it is perpendicular to the cornea; therefore, as the temporal probe is not perpendicular to the cornea it will exert less force as it hits the corneal surface. Force = mass x acceleration; therefore, as the mass of the probe is unchanged, less force will produce less deceleration as the probe rebounds, which will in turn register as a lower IOP by the RBT device. Variation in the peripheral corneal area hit by the probe may also be a factor (Muttuvelu et al., 2012).*

Fig. 43. *A photograph of the RBT probe, demonstrating the round profile of the tip. In addition, with a hand held device it is not always possible to stipulate an exact distance from the probe ball to the cornea and this may affect the result (Muttuvelu et al., 2012).*

Ideally the head of the probe should be held within 4 – 8 mm from the centre of the cornea. A limitation of this study was that the distance of the probe to the corneal surface was not accurately measured it was estimated and guided by markings on the trial frame situated on the child's face. Beasley *et al*., (2013) used a 5 mm distance as measured by the graticule on the trial frame, although no significant variation in IOP has been found with a probe distance from the corneal surface of 4, 6 or 8 mm (Takenaka *et al*., 2011). Histologically the centre of the cornea is thinner than the periphery with thinner collagen fibres which are more tightly packed at the centre giving it greater tensile strength and less elasticity (Fig. 8). Boote *et al*., (2003) hypothesise that as the less dense periphery is thicker due to an increase in collagen diameter and larger interfibrillar spacing and has lower tensile strength and greater elasticity, this may lead to lower IOP measurements. However, they do not state by how much. Chui *et al*., (2008) found that peripheral CCT was on average 41 µm thicker than central and the difference was not significantly correlated to the difference between peripheral and central RBT measurements, however their participants were healthy adults.

The difference between RBT_{off} and RBT_{on} was within ±1 mmHg in 41.2% of measurements, < 3 mmHg in 62% of measurements and ≥ 3 mmHg in 38% of measurements which is different from results in adult studies. Queiros *et al.,* (2007) found that 73.2% of temporal RBT values were within ±1mmHg of central RBT, 80% of nasal measurements were within ±1mmHg of central and 82.3% of temporal values were within ±1 mmHg of nasal. This may be due to lack of cooperation in children and inconsistent RBT positioning leading to less precision and accuracy. As far as the author is aware, this is the first study to look at suboptimal RBT in children with glaucoma and more research is needed to see how far out on the cornea reliable RBT measurements can be taken.

Most previous studies with the exception of Beasley *et al*., (2013) have compared RBT measurements taken 2 or 3 mm from the limbus of the cornea (Table 15). The majority have examined RBT in the nasal and temporal regions and some have examined the effect of angling the RBT probe from 5 to 10 degrees in the above positions (Takenaka *et al*., 2012; Muttuvelu *et al*., 2012; Beasley *et al*., 2013). Most RBT clinical measurements in adults are obtained from a central 4 mm zone (Beasley *et al*., 2013). However, readings in children may be less accurate due to Bell's phenomenon as described previously therefore it was decided

that RBT_{off} measurements should be measured further out at approximately 3 mm temporal to the centre of the cornea. Temporal measurements were chosen because they are closest those taken at the centre and have been shown to be a good substitute for central measurements (Yamashita *et al*., 2011).

All measurements were taken with the participant looking straight ahead. This is in accordance with other studies by Queiros *et al*., (2007); Takenaka *et al*., (2011); Muttuvelu *et al*., (2012) and Beasley *et al*., (2013). Yamashita *et al*., (2011) asked the participant to look up down or to the side to enable a more perpendicular measurement however this can result in an increase in IOP due to action of the extraocular muscles (Hofer *et al*., 1995). On the other hand, as previously noted the disadvantage of taking a measurement that is not perpendicular to the cornea is that this may reduce the force of the probe leading to an underestimation of the IOP (Fig.42). Also, asking participants to move their eyes during data collection is not always possible.

The order of RBT measurements and choice of eye were randomized where possible using a coin, by throwing it upwards and catching it to reveal heads or tails. This simple method was used because it enabled the participants to be involved where possible and it was very well received.

Several previous studies have selected the right eye (Muttuvelu *et al*., 2012; Beasley *et al*., 2013). However, in the present study right and left eyes were used because in some patients only one eye had glaucoma and was therefore selected. By using right and left eyes the random effects of the differences between the eyes was taken into account (Yamashita *et al*., 2011).

Study	Year	Temporal position	Mean (RBT_{off} - RBT_{on}) mmHg	Mean RBT (temporal) cornea vrs mean RBT (central)
Beasley *et al.*	2013	2 mm centre	N/A	No significant difference
Muttuvelu *et al.*	2012	2 mm limbus	-3 to 4	Significant difference
Yamashita *et al.*	2011	2 mm limbus	+1.6	No significant difference
Chui *et al*.	2008	3 mm limbus	-1.94	No significant difference
Queirós *et al*.	2007	2 mm limbus	-0.37	No significant difference

Table 15. *Results from previous adult studies.*

Mean RBT_{off} significantly over-estimated GAT by an average of 1.64 (SD 3.5) mmHg. The Wilcoxon Signed Ranks test revealed more ties between RBT_{off} and GAT than RBT_{on} and GAT demonstrating that more RBT_{off} measurements were closer to GAT than RBT_{on}. As previously noted in Experiment 1, correlation between RBT_{on} and GAT was strong; however, agreement between RBT_{off} and GAT was weaker especially at IOPs above 26 mmHg. By contrast Muttuvellu et al. (2012) found that peripheral RBT measurements significantly underestimate GAT however this was with RBT used as a fixed device, which resulted in larger IOP differences. Takenaka *et al*., (2011) found no significant difference between any corneal positions and GAT. Yamashita *et al*., (2011) found that temporal RBT measurements had the highest partial correlation coefficient with GAT and recommend that in cases where central corneal measurements cannot be obtained temporal values would be the closest. The present study agrees with this to some extent for IOP's up to 26 mmHg. However, a limitation of the present study was that suboptimal measurements were only taken temporally. Previous similar studies did not include children. Therefore, further research on the value of temporal RBT measurements in children is needed.

3.2.5 Conclusion

RBT_{off} was not significantly different to RBT_{on} and showed good agreement between 9 to 48 mmHg. Two thirds of RBT_{off} readings were within 2 mmHg of RBT_{on} and just under a half were within 1 mmHg therefore suboptimal measurements are useful clinically in children with glaucoma. However, this differs from adults where three quarters of these measurements are within 1 mmHg and it must be noted that one third of RBT_{off} differed by 3 mmHg or more from RBT_{on} with greater differences below 12 and above 40 mmHg. These measurements are within the range of intrasubject variability of ±4 mmHg according to Phelps and Phelps (1976). They are a useful guide but critical clinical decisions should not be based upon them. In addition, it was noted that the temporal RBT_{off} measurements may be closer to GAT than RBT_{on}, therefore the present study agrees with Takenaka *et al*., (2011) that temporal RBT readings seem to be a good substitute when central measurements cannot be obtained, however this needs further evaluation.

Dissemination

The results from chapter two were presented at three prestigious conferences: The United Kingdom and Ireland Society of Cataract and Refractive Surgeons (UKISCRS) 2017, the United Kingdom and Eire Glaucoma Society (UKEGS) 2018 and the United Kingdom Paediatric Glaucoma Society (UKPGS) 2019 and at a BWCH audit (2019).

3.3 Experiment 3: The association of co-existing conditions with the difference between suboptimal and central RBT measurements.

3.3.1 Methods

Where possible, the following co-existing characteristics of the participants from Experiment 2 were noted for this analysis: age, gender, ethnicity, ocular history, glaucoma treatment, strabismus, nystagmus, corneal defects, general health, refractive error (Rx), best corrected visual acuity (BCVA), axial length and corneal curvature.

When designing the study, the author considered measuring corneal curvature and central corneal thickness in addition to eye pressure measurements. However, only measurements that were routinely taken could be included in the design. It was also felt that extra measurements would not be practical as they would add to the participation time which might result in some of the children not completing.

3.3.2 Statistical analysis

A Fishers Exact test was used look at the relationship between the following co-existing characteristics in the cohort and the difference between RBT_{off} and RBT_{on}:

Age in years (group 1; 0-9, group 2; 10-15), gender (male/female), ethnicity (White, Asian, Mixed), ocular history (Primary congenital glaucoma, Secondary, Uveitic, Aphakic, Juvenile Open Angle Glaucoma), glaucoma treatment (medical, surgical, both), nystagmus (yes/no), strabismus (yes/no), corneal defect (yes/no), general health (good/co-morbidity), Rx in dioptres (group 1 ≤ 6.00, group 2 > 6.00) and BCVA in LogMAR (group 1 -0.16 to +0.,36, group 2 +0.38 to +1.80). RBT_{on} readings were subtracted from RBT_{off} and the resulting values (IOP status C) were divided into two groups: group 1 (< 3 mmHg), group 2 (≥ 3 mmHg).

3.3.3 Results

Characteristics of participants	N	%
Sample size	34	100
Gender		
Male	20	59
Female	14	41
Ethnicity		
White	21	62
Asian	12	35
Mixed	1	3
Age (years)		
Group 1 (0 – 9)	18	53
Group 2 (10 – 15)	16	47
General health		
Good	17	50
Co-morbidities	17	50
Strabismus		
Yes	17	50
No	17	50
Ocular history		
PCG	6	18
Other	8	23
Uveitic	4	12
Aphakic	13	38
JOAG	3	9
Treatment		
Medical	9	26
Surgical	2	6
Both	23	68
Nystagmus		
Yes	11	32
No	23	68
Corneal defect		
Yes	29	85
No	5	15
BCVA (LogMAR)		
Group 1 (-0.16 to +0.36)	17	50
Group 2 (+0.38 to +1.80)	17	50
Rx (dioptres)		
Group 1 (≤ 6.00)	15	44
Group 2 (> 6.00)	19	56

Table 16. *Characteristics of the study participants (glaucoma cohort): PGC= Primary Congenital Glaucoma, JOAG = Juvenile Open Angle Glaucoma. "Other" glaucomas include: SWS (1), traumatic (1), ARS (1), Peter's anomaly with penetrating keratoplasty (1), unclassified ASD (1), phacolytic with microphthalmia (1), congenital rubeosis iridis with microphthalmia (1) and microphthalmia (1). As there was insufficient data for central corneal thickness, corneal curvature and axial length, these values were disregarded.*

Age

To test whether proportions were different in each group a Fisher's Exact Test of probability was used with α = .05 as criterion for significance.

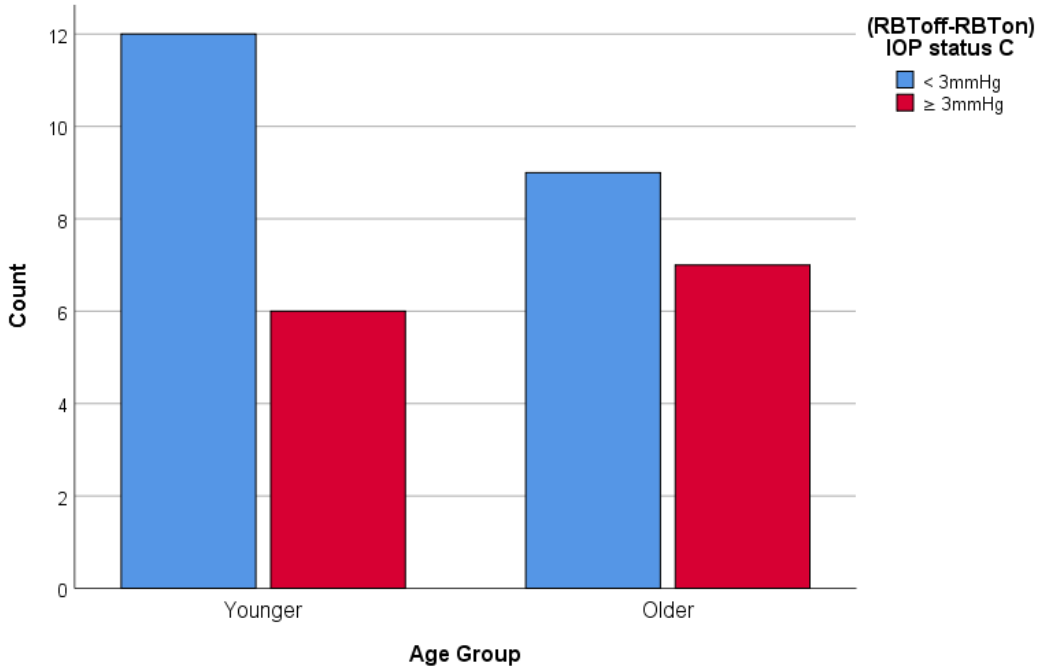


Fig. 44. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both age groups, with the lower age group having the greatest number. However, no significant association between age group and IOP status C, p = 0 .73 was found. Therefore, younger children are are not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3 mmHg or ≥ 3 mmHg than older children.*

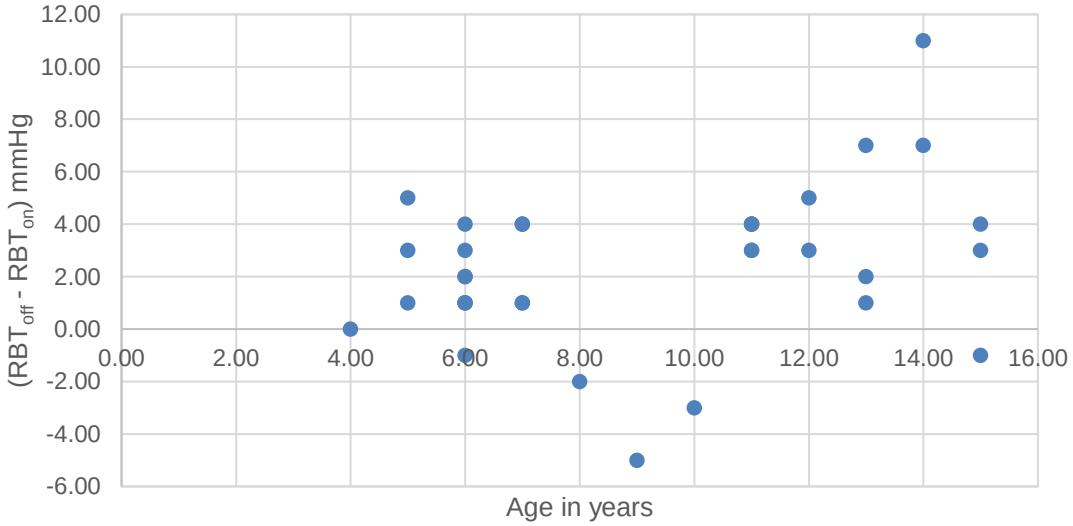


Fig. 45. *A scatter plot demonstrating that the difference between RBT_{off} and RBT_{on} increased until the age of 8 and was more spread out in children over 10 years. The majority of RBT_{off} measurements were either equal or lower than RBT_{on}. However, r^2 = 0.0353, which indicates that the difference between RBT_{off} and RBT_{on} was not dependent on age.*

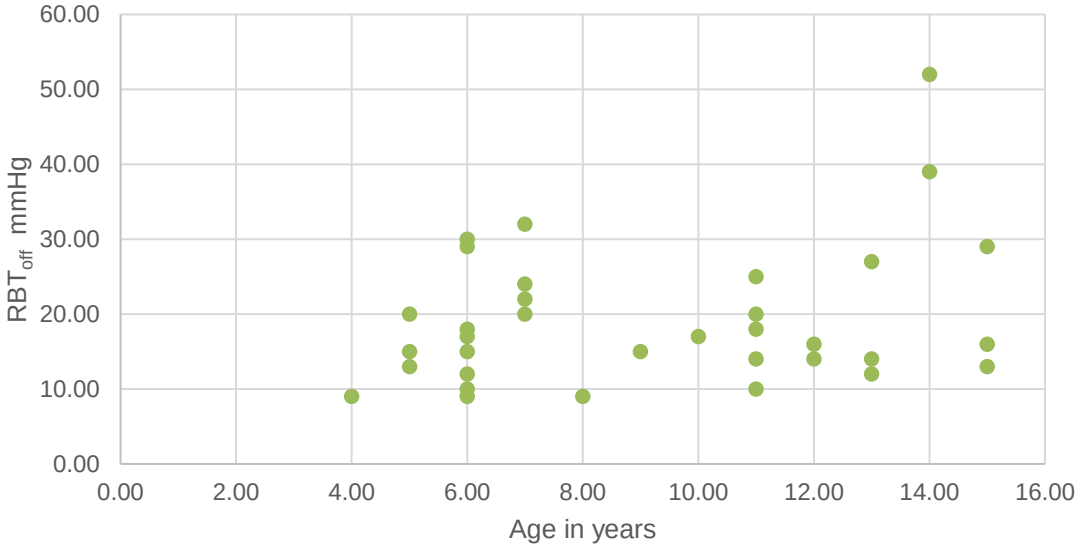


Fig. 46. *Demonstrates the overall increase of RBT_{off} with age in the participants. However, r^2 = 0.0762 which indicates that an increase in RBT_{off} was not dependent on age.*

Gender

Gender was divided into two groups (group 1.0: male, group 2.0: female). Fisher's Exact Test of probability was used to test whether proportions were different in each group with α = .05 as criterion for significance.

The average IOP (RBT_{on}) for males was 20 mmHg (SD 10.7) and for females this was 19.5 mmHg (SD 6.2), which was not significant.

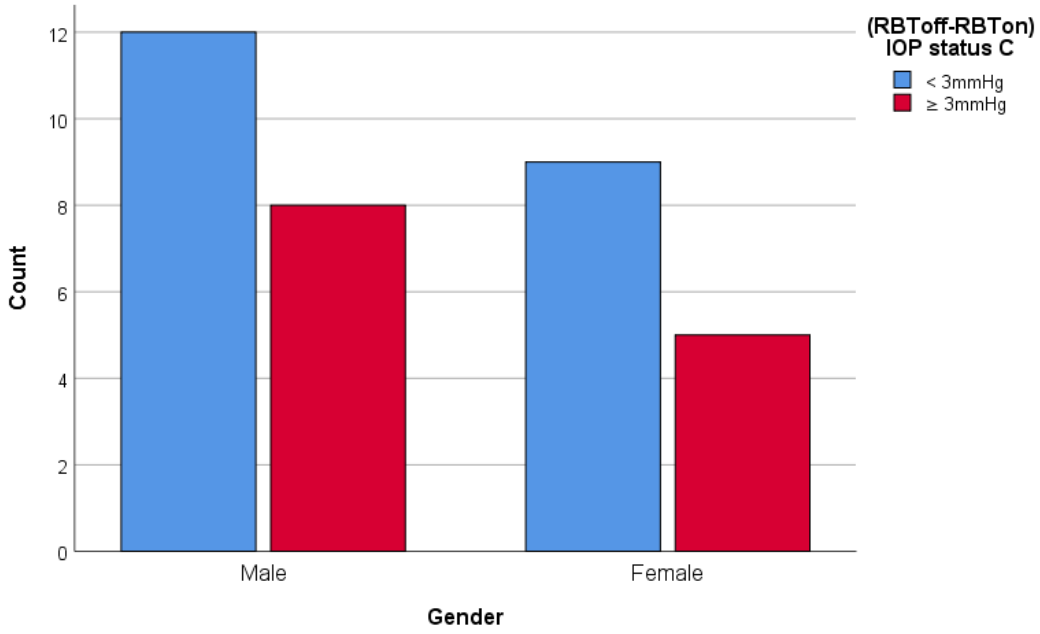


Fig. 47. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both groups, with males having the greatest number. However, no significant association between gender and IOP status C, p = 1.000 was found. This infers that males are not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg than females. Males: n=20, Females: n=14.*

Ethnicity

Ethnicity was divided into 3 groups (group 1 - White, group 2 - Asian, group 3 - Mixed). A Fisher's Exact Test of probability was not possible to compute in SPSS because this was a 3 x 2 table. To test whether proportions were different in each group a Chi-Square Test for independence was used with $\alpha = .05$ as criterion for significance.

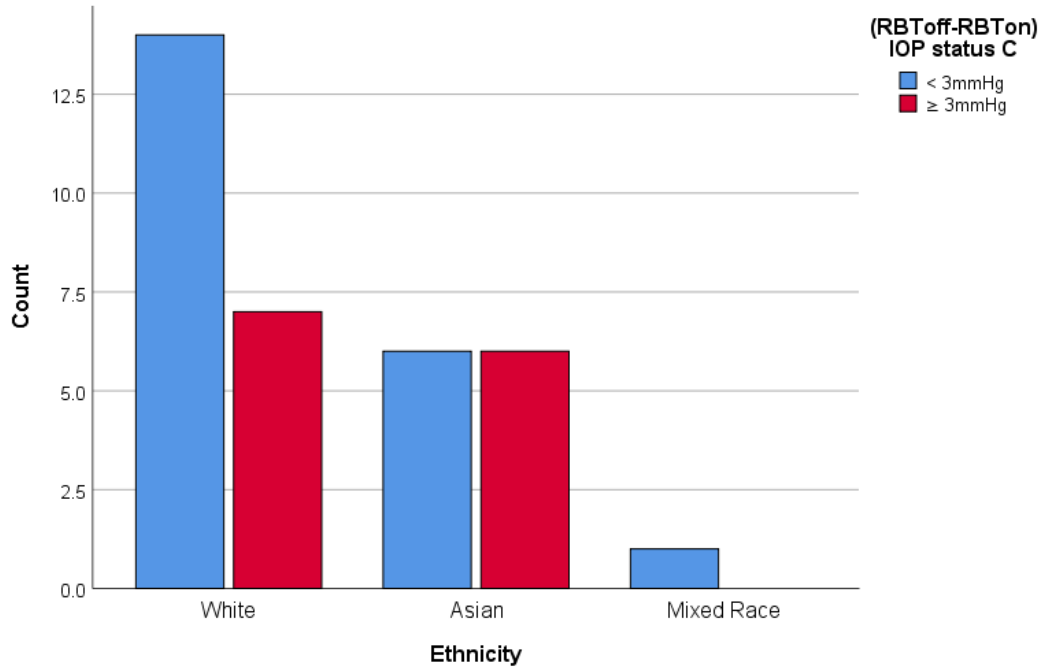


Fig. 48. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of the White group (n=21). No preference was shown in the Asian group (n=12). Group 3 did not contain enough data to be useful as it consisted of one participant who was Mixed (n=1). However, no significant association between type of treatment and IOP status C, $\chi 2$ (1, n = 34) = 1.536, p = 0.46 was found. However, this was a weak result because 50% of the cells had and expected count of < 5 so the assumptions for Chi-Sq were violated.*

Ocular history (type of glaucoma)

The types of glaucoma consisted of five groups:

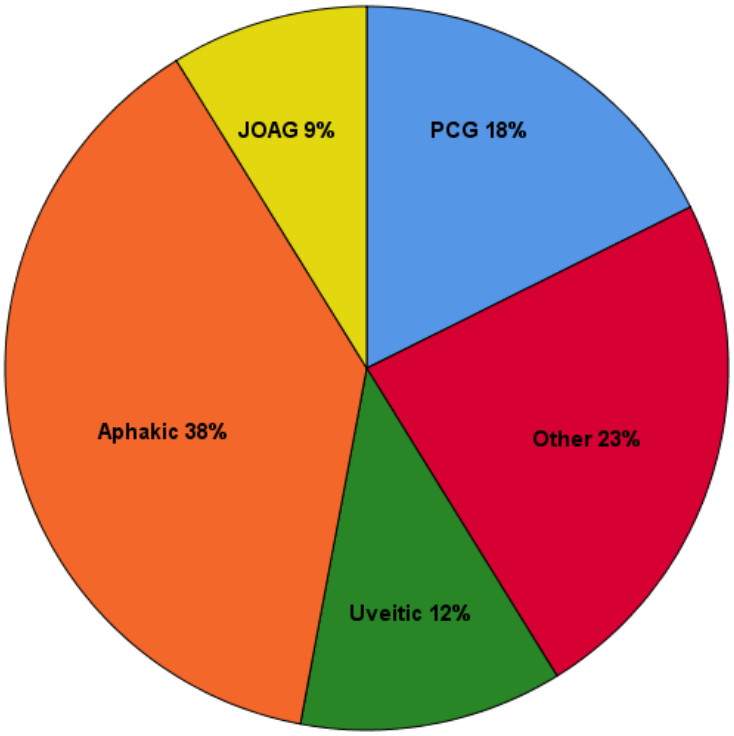


Fig. 49. *Pie chart demonstrating the representation of the different types of glaucoma in the participants. Glaucoma following cataract surgery (aphakic glaucoma) formed the largest group, followed by Other, which included glaucoma due to trauma and various syndromes and less common conditions (see Table 16). PCG (Primary Congenital Glaucoma) was responsible for just under 1/5 of glaucoma in the cohort. Uveitic and JOAG (Juvenile Open Angle Glaucoma) were less common.*

Fisher's Exact Test of probability was not possible to compute in SPSS because this was a 3 x 2 table. To test whether proportions were different in each group a Chi-Square Test for independence was used with α = .05 as criterion for significance.

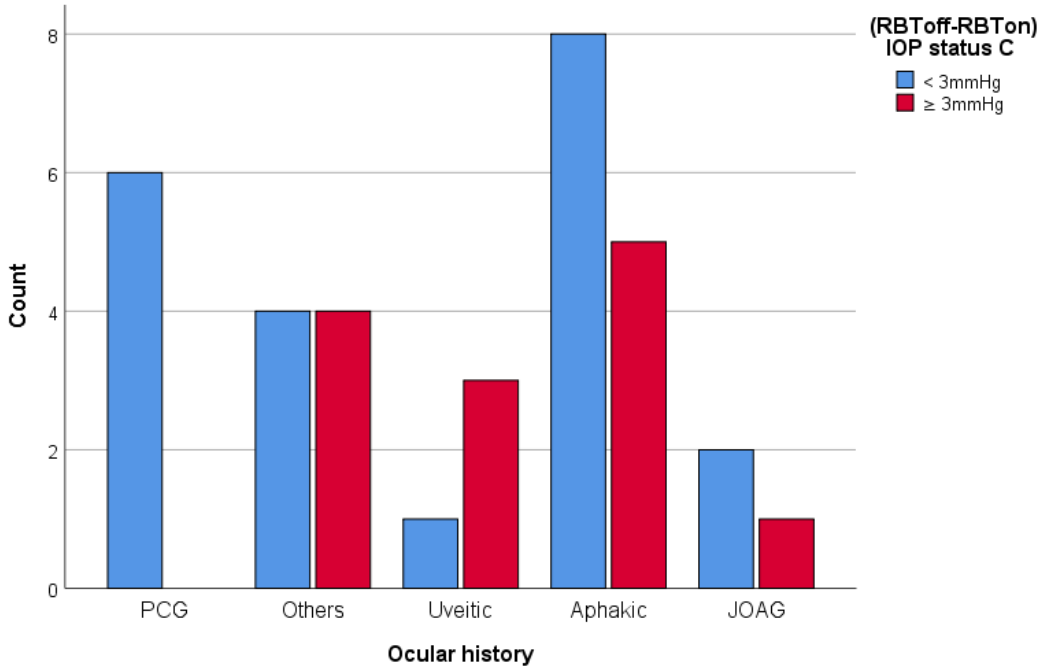


Fig. 50. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings was < 3 mmHg in the majority of the PGC, Aphakic and JOAG groups, showed no preference in the "other" group and were ≥ 3 mmHg in the majority of the uveitic group. However, no significant association between the type of glaucoma and IOP status C, $\chi 2$ (1, n = 34) = 6.503, p = 0 .17 was found. However, this was a weak result because 90% of the cells had and expected count < 5 so the assumptions for Chi-Sq were violated. PGC: n=6, Others: n=8, Uveitic: n=4, Aphakic: n=13, JOAG: n=3.*

Treatment

The type of glaucoma treatment consisted of three groups (group 1.0; medical, group 2.0; surgical, group 3.0; both medical and surgical). Fisher's Exact Test of probability was not possible to compute in SPSS because this was a 3 x 2 table. To test whether proportions were different in each group a Chi-Square Test for independence was used with α = .05 as criterion for significance.

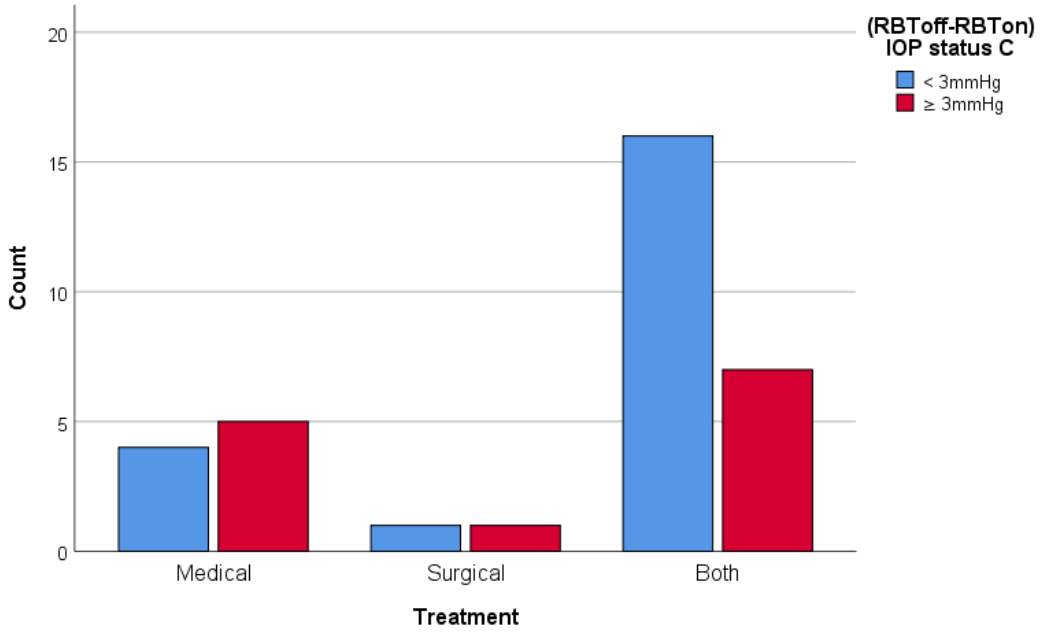


Fig. 51. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings was < 3 mmHg in the majority of the "both" treatment group 3.0, showed no preference in the surgical group 2.0 and were ≥ 3 mmHg in the medical group 1.0. However, no significant association between type of treatment and IOP status C, $\chi 2$ (1, n = 34) = 1.853, p = 0 .40 was found. This infers that the type of treatment is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg. However, this is a weak result because 50% of the cells had and expected count < 5 so the assumptions for Chi-Sq were violated. Medical: n=9, Surgical: n=2, Both n=23.*

Strabismus

The presence of strabismus was recorded as “yes” and the absence of strabismus was recorded as “no”. To test whether proportions were different in each group a Fisher’s Exact Test of probability was used with α = .05 as criterion for significance.

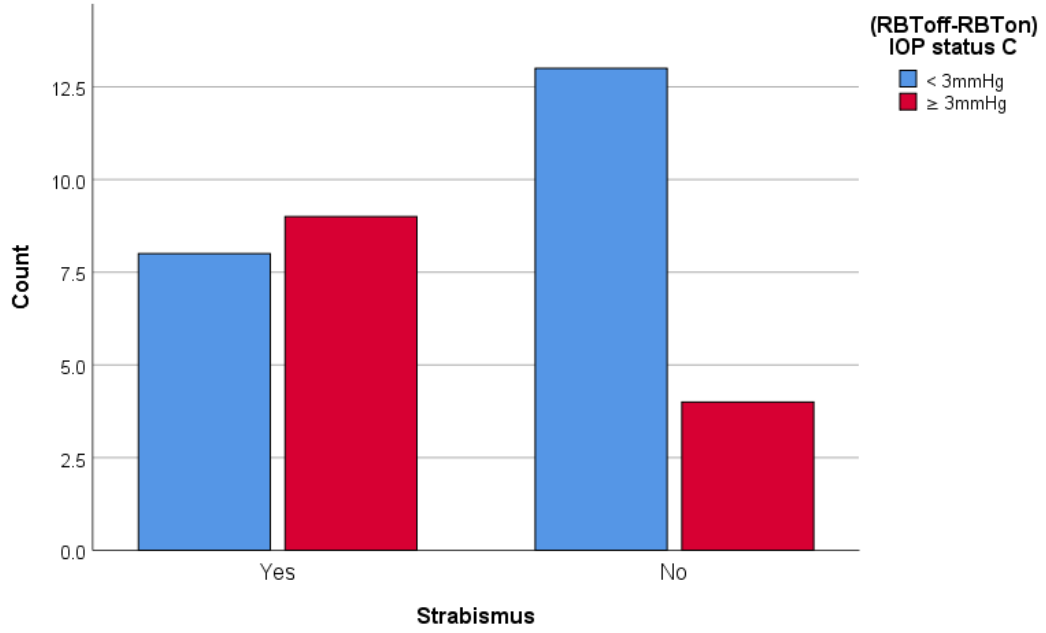


Fig. 52. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both groups, with the non-strabismic group having the greatest number. However, no significant association between Strabismus and IOP status C, p = 0.157 was found. This infers that strabismus not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3 mmHg or ≥ 3 mmHg than no- strabismus.Yes: n=17, No n=17.*

Nystagmus

The presence of nystagmus was recorded as “yes”, the absence of nystagmus was recorded as “no”. To test whether proportions were different in each group a Fisher’s Exact Test of probability was used with α = .05 as criterion for significance.

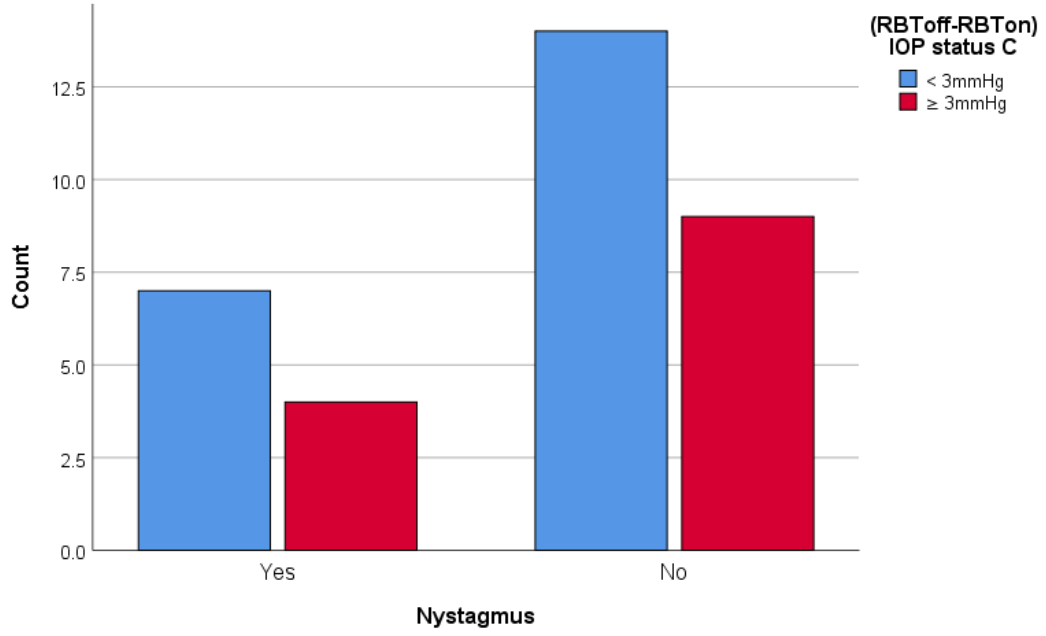


Fig. 53. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both groups, with the non-nystagmus group 2.0 having the greatest number. However, no significant association between nystagmus and IOP status C, p = 0 .16 was found. This infers that nystagmus is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3 mmHg or ≥ 3 mmHg than non-nystagmus. Yes: n=11, No: n=23.*

Corneal defect

The presence of a corneal defect was recorded as “yes”, the absence of a corneal defect was recorded as “no”. To test whether proportions were different in each group a Fisher’s Exact Test of probability was used with α = .05 as criterion for significance.

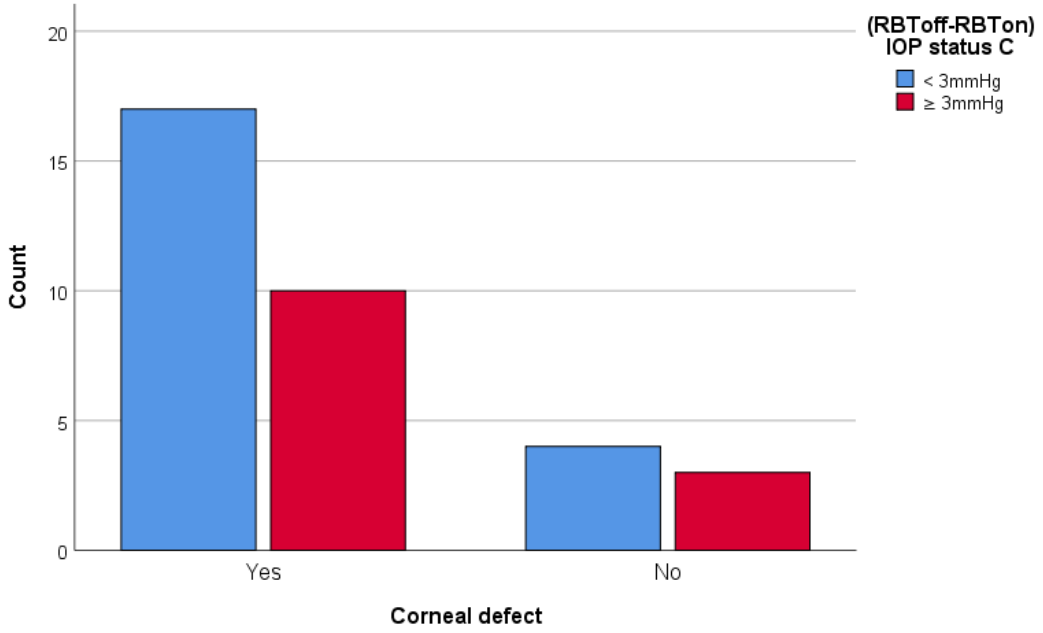


Fig. 54. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both groups, with the corneal defect group (yes) having the greatest number. However, no significant association between corneal defect and IOP status C, p = 1.000 was found. This infers that the presence of a corneal defect is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg than the absence of a corneal defect. Yes: n=29, No: n=5.*

General health

General health was divided into two groups, “good” and “co-morbidities”. To test whether proportions were different in each group a Fisher’s Exact Test of probability was used with α = .05 as criterion for significance.

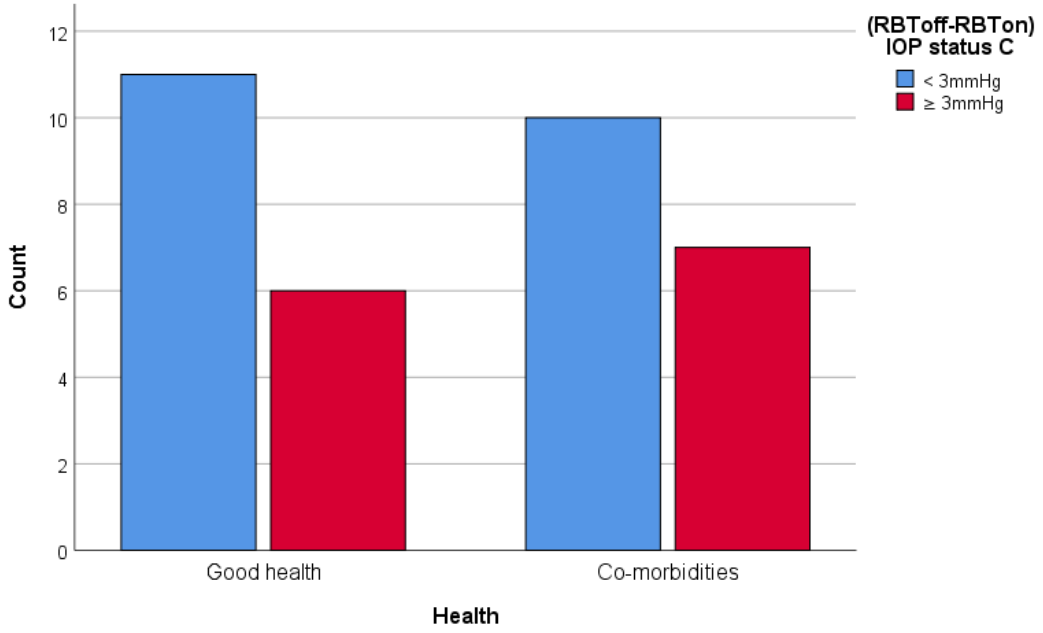


Fig. 55. *The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both groups, with the good health group having the greatest number. However, no significant association between general health and IOP status C, p = 1.00 was found. This infers that co-morbidity is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg than good health. Good health: n=17, Co-morbidities: n=17.*

Refractive error

Refractive error (Rx) was measured in dioptres and was divided into two groups (Group 1 ≤ 6.00, Group 2 > 6.00). A Fisher's Exact Test of probability was used to test whether proportions were different in each group, with α = .05 as criterion for significance. The difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both Rx groups, with the higher Rx group having the greatest number. However, no significant association between Rx and IOP status C, p = 0.73 was found. This infers that a higher Rx is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg than a lower Rx. (Rx = spectacle refraction in dioptres). Lower Rx: n=15, Higher Rx: n=19.

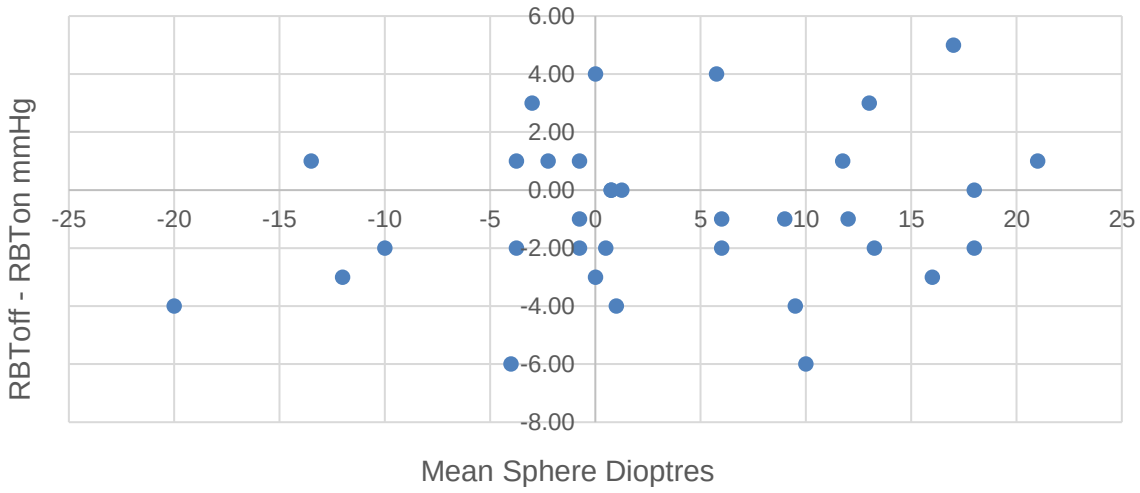


Fig. 56. *The scatter plot demonstrates that there was no correlation between (RBT_{off} – RBT_{on}) and mean spherical Rx (sphere + ½ cylinder), (y = 0.05x - 0.9352, r^2 = 0.0326).*

Best corrected visual acuity (BCVA)

This consisted of two groups and was measured in Log MAR units (group 1: good vision, g-0.16 to +0.36, group 2: poor vision +0.38 to +1.80 Log MAR). To test whether proportions were different in each group a Fisher's Exact Test of probability was used with α = .05 as criterion for significance. The difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both BCVA groups, with the good BCVA group having the greatest number. However, no significant association between best corrected visual acuity (BCVA) and IOP status C, p = 0 .48 was found. This infers that poor BCVA is not more associated with (RBT_{off} – RBT_{on}) measurements that are < 3mmHg or ≥ 3 mmHg than good BCVA. Good vision: n=17, Poor vision: n=17.

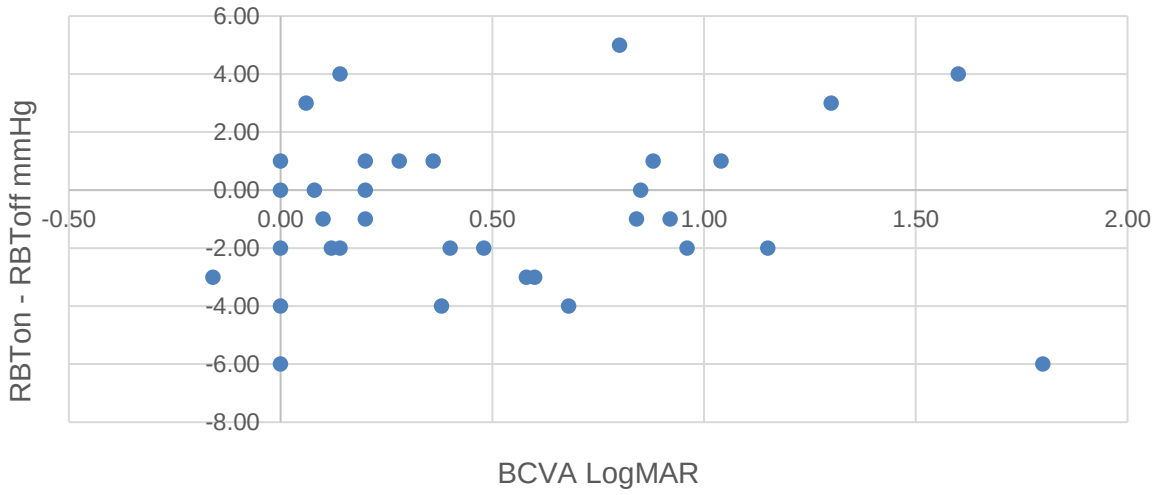


Fig. 57. *The scatter plot demonstrates that there was no correlation between (RBT_{off} – RBT_{on}) and BCVA (y = 0.5064x - 1.0176, r^2 = 0.0087).*

3.3.4 **Discussion**

The 34 participants recruited for experiments 1,2 and 3 were a heterogenous cohort. Table 16 demonstrates the different types of glaucoma, treatments, and co-morbidities present in this cohort. The high frequency of co-existing conditions in children with glaucoma has been noted before in a previous study by Flemmons *et al*., (2011b). Heterogeneity of participants and conditions can introduce bias and can be confounding (Miljanović *et al*., 2005). A limitation of the present study was that it was not possible to recruit children by type of glaucoma or treatment etc, within a reasonable time frame, due to the lack of available participants. The

factors that proved to be the most important in the present study were age, general health and treatment. Age was important because a wide age range of children were recruited with varying levels of cooperation and experience. General health was also an important factor because half of the participants had other general health conditions which influenced how well they felt on the day of participation. This in turn may have affected their level of cooperation. The participants were receiving different treatments for their glaucoma, either medical, surgical or both. Table 16 illustrates that the majority (68%) had experienced both medical and surgical treatments. Treatment was also an important factor. Surgical treatments varied from shunts to cataract surgery and medical treatments involved different types of drugs. Surgery resulted in corneal scars, but the majority were peripheral. Various glaucoma medications (e.g Brimonidine, Travaprost, Brinzolamide and Timolol) were used, all of which are associated with keratoepitheliopathy due to tear insufficiency and instability (Fraunfelder, 2006), which may have influenced IOP measurement (see treatment further on in this section).

It was not possible to analyse corneal curvature (CC) as not enough data was available. However, as the section on gender in this chapter explains, CC tends to be flatter in males than females (Eysteinsson *et al*., 2002). As previously described in section 1.8.7.2, CC can affect GAT measurements with steeper curves leading to higher readings. Liu and Roberts (2005) found IOP measured by GAT varied with a range of 1.76 mm Hg with variation of CC. In addition, as described in section 3.2.4, RBT may be affected by CC in that measurements that are not perpendicular to the corneal have been found to record lower IOPs. However, a probe misalignment of 10^{O} gives rise to an underestimation of < 1 mmHg, which is not clinically significant (Beasley *et al*., 2013).

Corneal hysteresis (CH) has been described in sections 1.6.3 and 1.8.5 and portrays the reaction of the cornea to fast deformation (Congdon *et al*., 2006). Low CH of 6 mmHg has been found in normal tension glaucoma patients (Kirwan *et al*., 2006). Touboul *et al*., (2008) found that lower CH (8 mmHg) had low correlation with IOP as measured by GAT (normal CH ranges from 8 to 15 mmHg).They also found that low CH is associated with underestimation of IOP by GAT and suggest that IOP should be checked by the ORA in such cases. Chui *et al*., (2008) found a significant negative correlation between RBT and CH ($r = - 0.67$, $P < 0.01$).

It is also interesting to note that Liu and Roberts (2005) used the RBT device in their study on corneal biomechanical properties and found that CH and CRF had more influence on IOP measurement than CCT.

Age

No significant association was found between the child's age group (1, 0 to 9 years; 2, 10 to 15 years) and (RBT_{off} - RBT_{on}) IOP status C (group 1 < 3 mmHg and group 2 ≥ 3 mmHg). Although the difference between RBT_{off} and RBT_{on} increased until the age of 8, was more spread out in children over 8 years and the majority of RBT_{off} measurements were ≤ RBT_{on}. This agrees with a study by González-Méijome *et al*. (2006) who found that peripheral RBT measurements were lower than central with the difference increasing with age. They found that central and temporal RBT had the strongest correlation in the middle-aged group (31 to 60 years), Spearman's r = 0.879, p < 0.001 and the least correlation in the younger age group (< 30 years) Spearman's r = 0.787, p < 0.001. Age was found to have an influence on peripheral corneal resistance and therefore an effect on rebound tonometry. González-Méijome *et al*. (2006) argue that this is not due to changes in corneal thickness, but due to changes in corneal biomechanical properties. However, the participants in their study were all adults over the age of 18 so this does not provide an exact comparison with the cohort in this study.

PEDIG (2011) found that CCT in children increases every year from 1 to 11 years, stabilizing thereafter. Therefore, as CCT can affect RBT measurements (Eysteinsson *et al*. 2002; ElMallah and Asrani. 2008) this change in thickness in the cornea may reflect changes in the difference between RBT_{off} and RBT_{on}, in addition to any biomechanical changes, in children under 11 years.

RBT_{off}, RBT_{on} and GAT all increased with age. Figure 46 shows the increase of RBT_{off} with age. However, the coefficient of determination $r^2 = 0.08$ illustrates a weak relationship between age and suboptimal RBT_{off} readings. R^2 was slightly higher for GAT versus age ($r^2 = 0.09$) and RBT_{on} versus age ($r^2 = 0.11$). However, these were also weak relationships (Figs. 35 & 36).

Gender

It was expected that gender may have an association with IOP status C, due to the differences between male and female corneas as outlined below. However, no significant association was found between the child's gender and IOP status C. Although, it is possible that any differences were masked by the heterogeneity of the participants. Interestingly, Figure 47 shows that the difference between RBT_{off} and RBT_{on} readings was < 3 mmHg in the majority of both gender groups. In addition, it is also interesting to note that males had the greatest number of (RBT_{off} - RBT_{on}) readings that were < 3 mmHg, which was expected due to the topographical differences between male and female corneas as discussed in the next paragraph.

The Reykjavik Eye Study used a random sample of 415 males and 510 females in a White population age 50 years and over and found the mean IOP of males, as measured by GAT, was 15.1 mmHg (SD 3.3), which was statistically significantly different from that of 15.8 mmHg (SD 3.1) found in females. They also found that the mean radius of curvature of the cornea was significantly flatter in males (7.78, SD 0.60 mm) than in females (7.62, SD0.58 mm), (Eysteinsson *et al*., 2002). This is important because it may explain the lower IOP readings found in males in their study, i.e. with applanation tonometry, more force must be applied to flatten a steeper cornea than a flat cornea which can lead to overestimation of IOP on the steeper corneas of females and underestimation on the flatter corneas of males (Whiteacre and Stein, 1993). The difference in corneal curvature between male and female corneas might explain the results of the present study, where (RBT_{off} - $RBT_{on)}$ measurements are closer in the flatter male corneas than the steeper female corneas. As previously discussed in section 3.2.4 the most accurate RBT measurements are taken perpendicular to the centre of the cornea. Measurements that are not perpendicular to the corneal surface suffer from a reduced rebound force and tend to record lower IOPs (Yamashita *et al*. (2011) and Queiros *et al*. (2007). However, these studies do not quantify this. As Figure 42 demonstrates, the probe was less perpendicular to the cornea in the periphery in the present study as the eye did not move position to enable a perpendicular measurement. It follows that the probe is more perpendicular to the cornea in flatter curves than in steeper. Therefore, flatter corneal curves may result in more accurate measurements that are closer to the optimal central ones.

Unfortunately, corneal curvature was not available for the majority of the participants in this study; therefore, it was not possible to investigate this further.

As previously discussed in section 1.8.8.2, RBT can be affected by CCT with thicker corneas resulting in a faster bounce off the cornea leading to higher recorded IOP. Eysteinsson *et al*., (2002) found that CCT was independent of gender and age in White adults. However, PEDIG found that girls had thinner corneas than boys with an average difference of approximately 5 µm ($p = .003$). Therefore, the differing CCTs between the genders may have influenced the results of this study, although it was not possible to investigate this further as insufficient CCT data was available. This may be something future studies could examine.

Average IOP has been found to vary between the sexes; The Barbados Eye Study, a large study of randomly selected 3752 predominantly black Barbadian participants without glaucoma aged 40 to 84 years, found that females had on average higher IOPs than males (Leske *et al*., 1997). Similarly, the Los Angeles Latino Study of the Latino population with Mexican ancestry found that females have a slightly higher, but not clinically significant, IOP difference compared with males (Memarzadeh *et al*., 2008). The Tehran Eye Study found mean IOP to be higher in females than males; however, after adjusting for co-existing health conditions (e.g. high blood pressure and diabetes), no significant difference was found (Hashemi *et al*., 2018). This agrees with the Beijing Eye Study which did not find an association between gender and IOP in the Chinese population (Xu *et al*., 2005). Similarly, the Blue Mountains Eye Study did not find a significant difference in IOP between males and females (Rochtchina et al., 2002). The present study only found a small difference between the average IOP (RBT_{on}) in males (20 mmHg, SD 10.6) and females (19.5 mmHg, SD 6.2). It is interesting to note that the males had a slightly higher mean IOP than the females when measured using RBT, which contrasts with the previous studies above who used GAT to measure IOP. However, the standard deviation was higher for males and the present study had a small sample size and the males and females were not equal in numbers, so this result may be unreliable.

Ethnicity

No significant association was found between the child's ethnicity (White, Asian or Mixed) and IOP status C. However, this was a weak unreliable result because the assumptions of Chi Square were violated (Fig. 48). The majority of participants in the present study were White (n=21), 12 were Asian and 1 was Mixed race (Black/White).

As previously discussed in 2.1.9.2, RBT is affected by CCT. Different ethnic groups have different mean CCTs which may in turn affect IOP measurement (Doughty *and Zaman*., 2000). Corneas tend to be thinner in adult Blacks than Whites (Nemesure *et al*., 2003). Shimmyo *et al*., (2003) found no significant difference in CCT between adult White, Asian and Hispanic populations. Fern *et al*. (2012) used data from young adults enrolled in the COMET study to examine CCT in the different ethnic groups and found that African-Americans have thinner corneas than Asians, Whites and Hispanics. PEDIG 2011 (children 0 -17 years old) found CCT in White and Hispanic corneas was not significantly different; however, Black subjects had significantly thinner central corneas (20 µm, P=.001) than Hispanic and White subjects of similar ages (PEDIG, 2011).

As far as the author is aware, the present study is the first to look at the association of ethnicity and suboptimal RBT measurements. The above result was not unexpected because most of the participants were White or Asian. Therefore, future larger studies with a wider ethnic base are needed.

Ocular history (type of glaucoma)

No association was found between the child's type of glaucoma and IOP status C was demonstrated, possibly due to the heterogeneity of the participants and the small sample size. Also, this was a weak unreliable result because the assumptions of Chi Square were violated. Figure 49 demonstrates the different types of glaucoma found in the cohort of the present study. Aphakic glaucoma was the largest group, followed by "others", PGC, uveitic and JOAG (Table 14). "Others" consisted of SWS, traumatic, ARS, Peter's anomaly, unclassified ASD, phacolytic with microphthalmia, congenital rubeosis iridis with microphthalmia and microphthalmia by itself. The difference between RBT_{off} and RBT_{on} was < 3 mmHg in the majority of the PGC, Aphakic and JOAG groups and ≥ 3 mmHg in the majority of the uveitic

group (Fig. 50). As the section on general health later on in this chapter explains, uveitic glaucoma is strongly associated with JIA, poor vision and can be difficult to treat (Sabri *et al*., 2008). The uveitic cohort generally had poor vision and therefore, poor fixation. However, this was also true of the PGC group, so the reason for this result remains unclear. Further studies are needed to further assess suboptimal RBT in children with uveitic glaucoma because as far as the author is aware, this is the first study to examine this.

Other syndromes such as ASD, ARS and Peters anomaly are caused by faulty prenatal gene transcription and have been described in section 1.5.2. ASDs can occur in conjunction with systemic diseases (described in the general health section of this chapter) or as isolated cases and are associated with a 50% risk of glaucoma (Reis and Semina, 2011). Cataract removal at a young age and microcornea are associated with the development of glaucoma (Biglan, 2006). PCG is an autosomal recessive disorder that results in high IOP in babies up to 12 months old, which can result in eye enlargement, optic nerve damage and blindness if untreated (Ali *et al*., 2009). JOAG develops in older children, is a more destructive form of glaucoma than adult POAG and can result in severe visual loss (Wiggs *et al*., 1996). All of these conditions have been described previously in sections 1.5.1, 1.5.2 and 1.5.3. It is also interesting to note that the traumatic glaucoma was the result of a car accident in which the child was travelling with an ill-fitting seat belt which damaged his eye on impact.

Reliable IOP measurement is important in all of these conditions because IOP is the one modifiable and prognostic factor available for the management of glaucoma (Coleman, 2012). As noted previously RBT has been enthusiastically adopted by Hospital Eye Departments due to its suitability of use with children (Dahlmann-Noor *et al*., 2013). Therefore, the results of this ground breaking study are important, as it useful to know that suboptimal RBT measurements take 3mm temporal to the corneal centre are clinically useful in children with glaucoma.

Treatment

No significant association was found between the child's type of treatment and IOP status C, although the result was weak and unreliable due to the assumptions for Chi-Sq being violated. Interestingly, the bar chart (Fig. 51). shows that (RBT_{off} - RBT_{on}) was < 3mmHg in the majority of the group that had experienced both surgery and medication. This was unexpected

because these were the participants who had experienced the most intervention. The exact reason for this is unclear. However, previous authors have shown that CH increases following IOP reduction by either surgery or prostaglandin medication (Sun *et al*., 2009; Agarwald *et al*., 2012).

The participants had different types of glaucoma with various treatments from medication to surgery or both. All of this can affect ocular rigidity which is known to affect IOP measurement by GAT, with higher measurements in areas of the cornea with calcification and lower in keratoconus and LASIK (Yamashita *et al*., 2011). Cataract surgery can temporarily alter corneal thickness peaking at 1 to 4 days post operation, returning to normal after 2 to 3 months, which can affect GAT readings (Doughty and Zaman, 2000).

Topical anaesthetics that are instilled for IOP measurement before GAT have been shown to reduce IOP (Almubrad and Ogbuehi, 2007). However, Dosunmu *et al*. (2014) found that this was not statistically significant. In addition, the use of muscle relaxants with general anaesthesia has been found to increase IOP (Youn *et al*., 1990). Diaz et al., (2008) compared IOPs measured by RBT and the Perkins tonometer and did not find any difference in the performance of RBT between patients treated with Travoprost 0.004% and healthy controls. Unfortunately, topical medication can cause discomfort and can be a burden for families to administer (Dahlmann-Noor *et al*., 2017).

As far as the author is aware, the present study is the first to examine the association between the type of glaucoma and suboptimal RBT measurements in children with glaucoma. A limitation of this study was the small sample size. Therefore, further larger studies are needed.

Strabismus

No significant association was found between the strabismic group and IOP status C (n = 34). This was unexpected because it was thought that there may be an association due to probe misalignment in the strabismic group affecting both RBT_{off} and RBT_{on} measurements (Fig. 42). The bar chart (Fig. 52) shows that the difference between RBT_{off} and RBT_{on} readings was less than 3 mmHg in the majority of both groups. The non-strabismic group had the greatest number which was expected because this group had the best probe alignment. Fifty percent

of participants had strabismus which is higher than normal populations. By contrast, the Baltimore Paediatric Eye Disease Study examined 3990 White and African American children and found a prevalence of < 2% in both populations (Friedman *et al*., 2009). A multi-ethnic study of young children in California USA aged 6 to 72 months found an incidence of strabismus of just over 3% in both Hispanic and Asian children (McKean-Cowdin *et al*., 2013). As far as the author is aware not much is known about the association of suboptimal RBT and strabismus in children with glaucoma. However, Flemmons *et al*. (2011b) did not find any association between RBT measurements that were higher than GAT and strabismus. Children with glaucoma present with more strabismus than the average population, therefore future studies are needed to examine the reliability of IOP measurement in these children.

Nystagmus

Nystagmus is an optokinetic involuntary movement of the eye in which the eye scans back and forth as it tries to stabilize a moving image (Maddess, 1984). It can be both monocular or binocular and is often associated with strabismus (Hertle, 2010).

It was expected that nystagmus may have an association with IOP status C and that the non-nystagmus group may not because the eye movements of nystagmus may cause probe misalignment which can result in less accurate IOP measurements. However, no significant association was found between nystagmus and IOP status C (n = 34). There is a paucity of information about the use of RBT in children with nystagmus. However, Flemmons *et al*. (2011b), did not find an association between nystagmus and (RBT-GAT) measurements. The bar chart shows that the difference between, RBT_{off} and RBT_{on} readings were less than 3 mmHg in the majority of both groups. However, as expected the non-nystagmus group did have the greatest number of RBT_{off} and RBT_{on} readings that were less than 3 mmHg (Fig. 53), possibly due to less probe misalignment. Children with congenital nystagmus often adopt a head posture to find the null point in order to reduce eye movements (Casteels *et al*.,1992), which may explain the lack of association between nystagmus and IOP status C. As far as the author is aware this is the first study to examine suboptimal RBT in children with glaucoma and nystagmus; therefore, further research is needed.

Corneal defect

IOP measurement can be difficult in patients with corneal pathology due to associated scars and irregularity of the corneal surface (Kaufman *et al*.1970). Irregular corneas can cause fluorescein pooling and distorted mire images with GAT resulting in unreliable results (McMillan and Forster, 1975). Corneal pathologies that can affect IOP measurement include bullous keratopathy, keratoplasty, keratoconus, corneal dystrophies, corneal scars due to surgery (glaucoma, penetrating trauma, cataract), Haab striae in PCG, Axenfeld-Rieger syndrome and Peters anomaly (Rosentreter *et al*. 2013).

The majority of the corneal defects in this study were small peripheral scars caused by cataract surgery. However, one had full thickness PK due to Peters anomaly and three had microcornea.

It was expected that no association would be found between corneal defects and IOP status C because the majority of the defects were peripheral and did not affect the area of the cornea used in this experiment. Indeed, no significant association was found between the presence of a corneal defect and IOP status C (n = 34). Interestingly, the bar chart in Figure 54 shows that the difference between RBT_{off} and RBT_{on} readings were less than 3 mmHg in the majority of both groups. However, unexpectedly the “corneal defect” group had the greatest number of (RBT_{off} - RBT_{on}) readings that were less than 3 mmHg. This may be an anomaly due to the small sample size.

Mc Millan and Forster (1975) found a statistically significant difference between normal and oedematous corneas of owl monkeys at 20, 30 and 40 mmHg levels of IOP and between Perkins and GAT in these subjects. Azuara-Blanco *et al*. (1998) evaluated IOP measurements in patients with band keratopathy and glued corneas and found that it was not possible to obtain GAT measurements in 3 of the 15 subjects due to corneal irregularity. Ton-Pen readings from unaffected areas were obtained; however, it was noted that Ton-Pen measurements taken from the oedematous areas were higher due to the hardness of the calcium plaques on the surface of the cornea. The RBT can be used in a similar way to the Ton-Pen to obtain measurements from unaffected areas of the cornea due to its small footprint. Rosentreter *et al*. (2013) examined 171 eyes with corneal pathology and found that it was not possible to measure IOP by GAT and DCR in all of the participants. However, it was

possible using RBT and RBT significantly underestimated IOP when compared with GAT and DCT. An important finding of the present study was that suboptimal RBT temporal measurements taken 3 mm form the corneal centre were not statistically different to central measurements (See chapter 3.2.3). This demonstrates the usefulness of RBT for IOP measurement in corneas with scars and other pathology.

General health

The association between general health and IOP status C was examined because it was thought that children with underlying health conditions may not be as cooperative as healthy ones when participating in the study. However, no significant association was found between general health and IOP status C (n = 34). Although the result was weak and unreliable due to the assumptions for Chi-Sq being violated

The bar chart shows that the difference between RBT_{off} and RBT_{on} readings were less than 3 mmHg in the majority of both groups with the healthy group having the greatest number (Fig. 55). This was not unexpected because healthy participants were more able to cooperate. Previous studies have found an association between IOP and general health. The Beaver Dam Eye Study (1992) was a large study of 4926 adults in the United States of America. The authors found that raised IOP is associated with blood pressure, body mass index (BMI), diabetes, cholesterol levels and pulse (Klein et al., 1992). Nomura *et al*. (2004) examined 1855 Japanese adults and also found a significant association between IOP and hypertension and BMI. The Blue mountains Eye Study of 3654 adults in Australia also found an increased prevalence of glaucoma in participants with diabetes (Mitchell *et al*., 1997).

Examples of co-existing health conditions can be seen in Table 17. Uveitic glaucoma is a secondary inflammatory glaucoma that is associated with JIA (Kanski, 1990) and has been discussed in section 1.5.2. All of the participants in the present study with uveitic glaucoma also had JIA. The participants with ARS and the un-named ASD both had heart defects which are known to occur with the failure of development of the anterior chamber (Sowden, 2007). In addition, the participant with ARS had hearing loss, which is also associated with this syndrome (Idrees et al., 2006). Participants with aphakic glaucoma had a range of general

health conditions from asthma to epilepsy, which were not necessarily associated with their eye condition.

Type of glaucoma	Co-existing health problem
Un-named ASD	Pulmonary valve stenosis
JOAG	Diabetic 1
JOAG	Asthma
Uveitic	JIA
Aphakic	Asthma
Aphakic	Seizures
Aphakic	Peroxisomal disorder
ARS	Heart valve and ear problems
PGC	Heart murmur

Table 17. *Examples of the types of glaucoma found within the cohort and their co-existing general health condition. Fifty percent of participants in this glaucoma cohort had other general health conditions. It is also important to note that 50% of the cohort had a range of glaucoma from PCG to aphakic but were otherwise healthy.*

Quality of life can be significantly affected by childhood glaucoma, which can exert a considerable impact on the family and can be likened to the stress of having a congenital heart defect, liver transplant or acute lymphoblastic leukaemia (Dahlmann-Noor *et al*., 2017). So, it was not surprising to find that one potential participant with EDS in the present study was unable to take part because he was not feeling up to it.

Refractive error

As far as the author is aware, there is a paucity of information about the association suboptimal RBT measurements and refractive error generally and very little is known about this in children with glaucoma. The present study found no significant association between refractive error groups measured in dioptres (group 1, ≤ 6.00; group 2, > 6.00) and IOP status C (n = 34). There were 15 children in refractive error group 1 and 19 in refractive error group 2, which demonstrates that the majority of children with glaucoma in the present study had refractive errors of > 6D.

These results agree with Yamashita *et al*. (2011), who looked at 102 healthy adults and found no association between spherical refractive error and the difference between RBT_{on} and temporal RBT taken 2 mm from the limbus.

The bar chart shows that the difference between RBT_{off} and RBT_{on} was less than 3 mmHg in the majority of both Rx groups (Fig. 48). Unexpectedly, the higher Rx group had the greatest number. However, the sample size was small. Therefore, this may be an anomaly.

Differences in corneal biomechanics have been found to be associated with refractive error, with high myopia associated with flatter corneas and lower levels of CH. By contrast, hypermetropia is associated with steeper corneas and higher CH (Bueno-Gimeno *et al*., 2014). As previously discussed in section 3.2.4 of this chapter, an increase in corneal curvature can lead to underestimation of RBT_{off} when compared with RBT_{on} due to angle of the probe as it bounces off the cornea. Therefore, one might expect RBT_{off} and RBT_{on} to be more similar in myopes than hyperopes as myopes have flatter corneas. However, corneal biomechanical factors such as CH and CRF are also known to affect RBT (Chui *et al*., 2008) and differ between refractive errors as noted above.

Refractive error has been found to be correlated to the difference between RBT and GAT. Avitabile *et al*. (2010) examined a group of 327 healthy adults with myopia, hypermetropia and astigmatism and found that RBT was significantly higher than GAT in all participants ($p < 0.000$). However, 34.5% of myopes had a difference of > 2mmHg compared with 13.3% of hyperopes, 7.6% of astigmats and 17.9% of emmetropes. They found that the difference increased at high IOPs ($p < 0.001$).

IOP has also been found to be related to refractive error in children. PEDIG (2011) noted that CCT was 1 µm thinner and IOP 1.5 mmHg higher for every dioptre increase in myopia ($p < .001$). Likewise, Nomura *et al*. (2004) found a positive association between IOP and increase in myopia in their study of adults aged 40 to 82 years. After adjusting for age and CCT they found that IOP in moderate myopia was significantly higher than hypermetropia.

A limitation of the present study was that refractive error was divided into two groups which was necessary for analysis. The groups were divided by spherical power, unfortunately it was not possible to allow for astigmatism.

Future studies are needed to examine the effect of astigmatism on RBT measurement.

BCVA

No significant association was found between BCVA measured in LogMAR units (group 1, -0.16 to +0.36; group 2, 0.38 to +1.80) and IOP status C (n = 34). However, the bar chart shows that the difference between RBT_{off} and RBT_{on} readings were < 3 mmHg in the majority of both BCVA groups. The lower BCVA group had the greatest number, which was expected because this group had the best visual acuity and could therefore fixate better during RBT measurement. There were 17 children in each group. Group 1 consisted of children with reasonable visual acuity and group 2 consisted of children with moderate to very poor, which demonstrates the devastating effect of childhood glaucoma on vision. One half of the participants with uveitic glaucoma had poor vision, however, the sample size was too small to draw any conclusions. This partly agrees with Thorne *et al*. (2007) who found that uveitic inflammation was associated with an increased risk of visual loss (relative risk = 2.02, p = .006). The participants with PGC and Aphakic glaucoma also had poor vision (PGC: 0.00 to 0.36 LogMAR; Aphakic: 0.00 to 1.3 LogMAR). The best BCVA was found in the JOAG group (0.00 to 0.10 LogMAR)

Amblyopia and glaucomatous optic nerve damage are often responsible for poor vision in children with glaucoma (Kargi *et al*., 2006). Other causes are media opacities, anisometropia and irregular corneal astigmatism (Morin and Bryars,1980). Control of IOP is the most important way to prevent vision loss in these patients (Kargi *et al*., 2006).

A limitation of this study as far as examining the BCVA results was the small sample size. It was not possible to divide the BCVA results into more than two groups. As far as the author is aware the present study is the first to examine the association of BCVA and suboptimal RBT measurements in children with glaucoma. Therefore, future studies with larger sample sizes are needed.

3.3.5 Conclusion

The above co-existing conditions were not associated with the difference between RBT_{off} and RBT_{on} measurements when RBT_{off} was taken 3 mm from the corneal centre; however, the difference between RBT_{off} and RBT_{on} was greater in children above 8 years old. RBT_{off} was more often closer to RBT_{on} in male children than females, possibly due to males having flatter

corneas. However, the genders did not have an association with (RBT_{off} - $RBT_{on)}$. Interestingly, corneal scars were not found to associate with (RBT_{off} - $RBT_{on)}$ measurements; however, the majority of the corneal scars in this study were peripheral. Central scars may have given a different result and warrants further investigation. Topical glaucoma medication has been shown to effect IOP measurement in previous studies but did not associate with (RBT_{off} - $RBT_{on)}$ measurements in this study.

The present study also demonstrates that children with glaucoma often have high refractive errors, poor vision, strabismus and nystagmus, all of which can make IOP measurement more challenging. They can also have associated general health conditions which can affect their quality of life. When combined with the results from Experiment 2, the results from Experiment 3 further indicate the clinical usefulness of suboptimal RBT_{off} measurements when taken temporally 3 mm from the centre of the cornea in children with glaucoma.

Dissemination

The results from chapter 3 were presented at two prestigious conferences: UKEGS (2018) and UKPGS (2019) and at a BWCH audit (2019)

Chapter 4

The reliability of suboptimal "off-axis" RBT measurements in children with Heritable Connective Tissue Disease.

4.0 Experiment 4

4.1 Introduction

Children with HCTDs need to have their IOPs measured due to the associated ocular complications and risk of developing glaucoma. Cross and Jenson (1973) found that glaucoma was present in 7.7% of patients with ectopia lentis (EL) and 14.8% of aphakic eyes in patients with MFS. Maumenee (1981) found that EL was associated with an increase in axial length and decrease in corneal curvature which can be a result of stretching of the sclera which can lead to breakage of the lens zonules. Izquierdo *et al*., (1992) noted the following types of glaucoma were present in patients with MFS in descending order of magnitude:

1. Open-angle glaucoma
2. Acute angle-closure
3. Phacolytic glaucoma
4. Neovascular glaucoma
5. Post-lens extraction
6. Post-scleral buckling procedure

By far the vast majority were secondary open-angle glaucoma with both sexes equally affected. The age range was 1 to 79 years with a mean age of 29.6 years. In this study, open angle glaucoma is described as secondary in nature due to the structure of the anterior chamber angle of MFS patients. Iris processes, strands and fibres may be present, the ciliary muscle may be underdeveloped, and longitudinal muscle fibres pass internally and in front of the scleral spur, all of which can reduce aqueous outflow leading to an increase in IOP and the onset of glaucoma (Wachtel, 1966).

Maumenee (1958) proposed that ciliary muscle contraction closes Schlemm's canal and flattens the trabecular meshwork resulting in the slow exit of aqueous humour leading to raised IOP and glaucoma. In addition, defects in connective tissue associated with MFS may result in a weakened lamina cribrosa and an increased risk of nerve fibre damage at the optic

nerve head. Secondary vascular abnormalities may lead to reduced blood flow to the eye which can also result in an increased risk of glaucomatous damage (Hardin, 1962).

EL and the vitreous can result in pupillary block which leads to raised IOP. The lens can dislocate into the anterior chamber spontaneously as a result of trauma or during pupillary dilation. EL can lead to partial closure of the anterior chamber angle, leading to chronic angle-closure glaucoma. Although defective zonules can lead to increased lens thickness and curvature, primary angle closure glaucoma is not associated with MFS where corneas are flat, anterior chamber depth is deep and axial length can be longer than average (Izquierdo *et al*., 1992). By contrast eyes with primary angle closure glaucoma have steep corneas, shallow anterior chambers and short axial length. EL can also result in subluxation posteriorly into the vitreous where membranes may develop joining the lens to surrounding structures. Mild inflammation and moderate raised IOP may develop due to phagolysis. Secondary glaucoma can occur following lens extraction and retinal detachment surgery which may be associated with inflammation and pigment release (Izquierdo *et al*., 1992).

EDS is a generalized heritable connective tissue disorder usually but not always dominantly inherited and has been described in section 1.7.1. Ocular manifestations include epicanthal folds, strabismus, myopia, EL and retinal detachment. Patients with EDS may have hypermobile joints, cutaneous hyper-extensibility, tissue fragility and thin corneas which can lead to easy rupturing of the globe through trauma (Beighton, 1970). Pesudovs (2004) found that the cornea of an EDS type 1 patient was 360 μm in the centre and 370 to 438 μm in the mid peripheral cornea. However even though the thin cornea with defective collagen affected the biomechanics of the cornea, the surface topography was normal with no ectasia.

IOP measurement in children with HCTD is subject to the same difficulties as described previously in section 3.2.4 with eye movements leading to suboptimal RBT measurements. As far as the author is aware nothing is known about the reliability of suboptimal RBT measurements in children with HCTDs who have defective collagen or fibrillin. Therefore, this ground breaking study addresses this.

4.1.1 Methods

Following assent/consent a total of 16 children aged between one month and sixteen years of any race or gender were recruited from children attending the Eye Department of Birmingham Children's Hospital for routine appointments. Eight of these children had HCTDs and eight were healthy controls with no eye disease (no eye surgery/medication, no high spectacle prescription i.e < +/- 6.00 DS and ≤ -2.00D astigmatism).

Exclusion criteria were as follows:

- Participants unwilling to have Goldman Applanation Tonometry and /or rebound tonometry.
- Participants with a known allergy to either proxymetacaine or fluorescein eye drops.
- Pregnancy
- Premature birth (babies up to 1 year old)
- High spectacle prescription in eyes with no disease (> +/- 6.00 DS and > -2.00D astigmatism)

The protocol followed was the same as Experiments 1 and 2. i.e Following assent/consent RBT_{on} measurements were taken at the centre of one eye and RBT_{off} measurements were taken 3 mm temporal to RBT_{on}, using the monocular pupillary distance indicated by trial frame on the participants face. Following this a GAT measurement was taken on the same eye. In addition, the following characteristics were noted; type of HCTD, age, gender, BCVA, Rx and presence or absence of EL was recorded.

4.1.2 Statistical analysis

The sample size for the Experiment 4, with a case control study design, was calculated using a *priori* power analysis for an ANOVA: repeated measures within- between interaction with a power of 95%, an effect size of 0.5 and an alpha level 5%. This indicated a total sample size of sixteen, comprising of eight children from each of the two groups. A Mixed-Factor Two-Way Repeated Measures analysis of variance was conducted to assess the impact of a participant's condition (HCTD or healthy controls) on the difference between IOP measured by

the rebound tonometer 3 mm temporal to the centre of the cornea (RBT_{off}) and at the geometric centre of the cornea (RBT_{on}).

The HCDT group consisted of a mixture of HCTDs. Therefore, in addition, a Mixed-Factor Two-Way Repeated Measures analysis of variance was repeated as above for the following groups:

1. sMFS HCTD group (without the nMFS and hEDS data) (6 participants).
2. MFS + nMFS group (without the hEDS data) (7 participants).

Both of these groups were age matched with the healthy controls.

A Mixed-Factor Two-Way Repeated Measures analysis of variance was also conducted for RBT_{on} and GAT (both had normal distributions).

4.1.3 Results

Eight eyes from children with HCDT and 8 from healthy controls were included. RBT_{off} measurements in the HCDT cohort ranged from 10 to 20 mmHg with a mean of 16.3 (SD 3.1) mmHg. RBT_{on} in the same cohort ranged from 11 to 22 mmHg with a mean of 18.1 (SD 3.5) mmHg (Table 18). RBT_{off} measurements in the healthy controls ranged from 10 to 22 mmHg with a mean of 16.3 (SD 3.9) mmHg and RBT_{on} in the same group ranged from 11 to 24 mmHg with a mean of 17.1 (SD 4.2) mmHg (Table 19).

No significant interaction was found between the participant's condition and IOP, Wilks' Lambda = 0.95, F (1, 14) = .767, p = .396, partial η^2 = .052. There was no significant main effect for IOP, which suggests that the difference between RBT_{off} and RBT_{on} was not significantly affected by the participants condition, although mean RBT_{off} was lower than mean RBT_{on} in both groups. The connective tissue group showed a greater mean difference (-1.88 (SD 1.7) mmHg p > .05) than the controls (-0.75 (SD 3.2) mmHg p > .05). However, the controls had a larger standard deviation. Wilks' Lambda = 0.77, F (1, 14) = 4.177, p = .060, partial η^2 = .230. Levene's Test of Equality of Error Variances: RBT_{off} p = 0.399 and RBT_{on} p = 0.637(Fig. 58).

HCTD	Minimum	Maximum	Mean	SD
RBToff	10.0	20.0	16.3	3.1
RBTon	11.0	22.0	18.1	3.5

Values are in mmHg

Table 18. *Descriptive statistics for the whole HCTD cohort, RBT_{off} and RBT_{on} (n = 8).*

Controls	Minimum	Maximum	Mean	SD
RBToff	10.0	22.0	16.3	3.9
RBTon	11.0	24.0	17.1	4.2

Values are in mmHg

Table 19. *Descriptive statistics for age matched controls, RBT_{off} and RBT_{on} (n = 8).*

HCTD	Age	Gender	Spectacle Rx	BCVA	EL
MFS	5	F	-3.00/-6.00 x 180	0.54	Yes
nMFS	6	F	-5.50	0.46	Yes
MFS	6	M	+5.00/-9.00 x 180	0.84	Yes
hEDS	6	M	+1.50/-0.50 x 175	0.04	No
MFS	8	F	-11.00	0.55	Yes
MFS	10	F	-4.00/-7.50 x160	0.10	Yes
MFS	10	F	+17.00	0.1	Yes
MFS	15	M	-9.00/-1.00 x180	0.18	No

Table 20. *Results table demonstrating the characteristics of the HCTD cohort with age range (years), gender, spectacle prescription (Dioptres), BCVA (LogMAR) and presence of ectopia lentis (EL). nMFS = non Marfan Syndrome fibrinopathy, hEDS = hypermobile Ehlers Danlos Syndrome.*

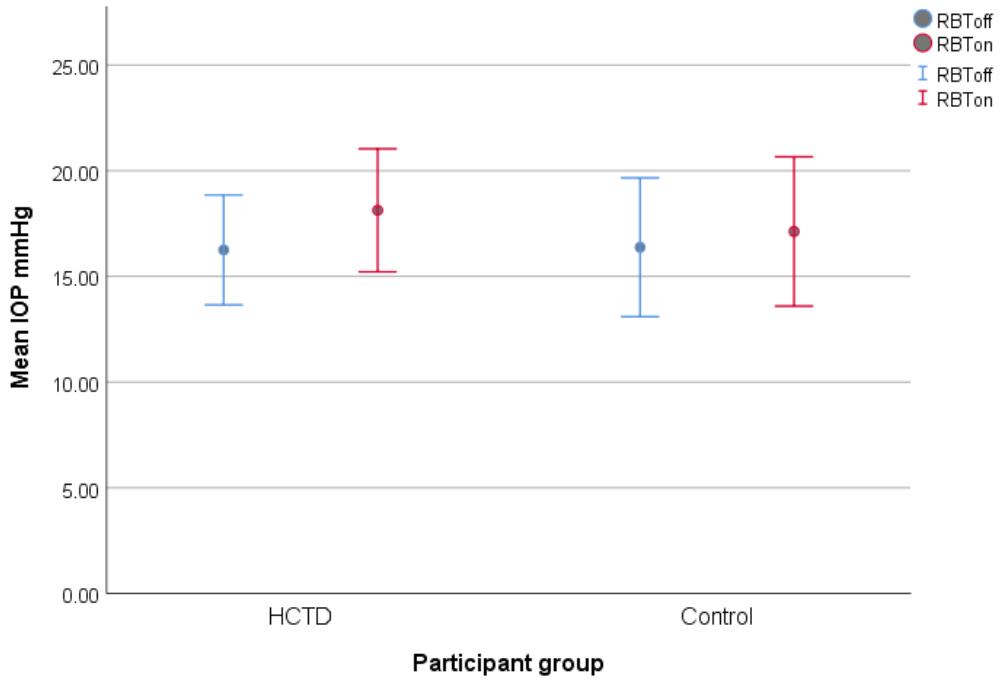


Fig. 58. *Graph showing mean RBT_{off} and RBT_{on} IOPs measured for the whole HCTD and control groups with 95% CI error bars. The graph demonstrates that although the difference was not significant, a larger difference was found between RBT_{off} and RBT_{on} in the HCTD group (-1.88 (SD 1.7 p > .05) mmHg than the controls (-0.75 (SD* 3.2 p > .05) *mmHg). However, the larger standard deviation found in the controls indicates a wider range of readings in this group.*

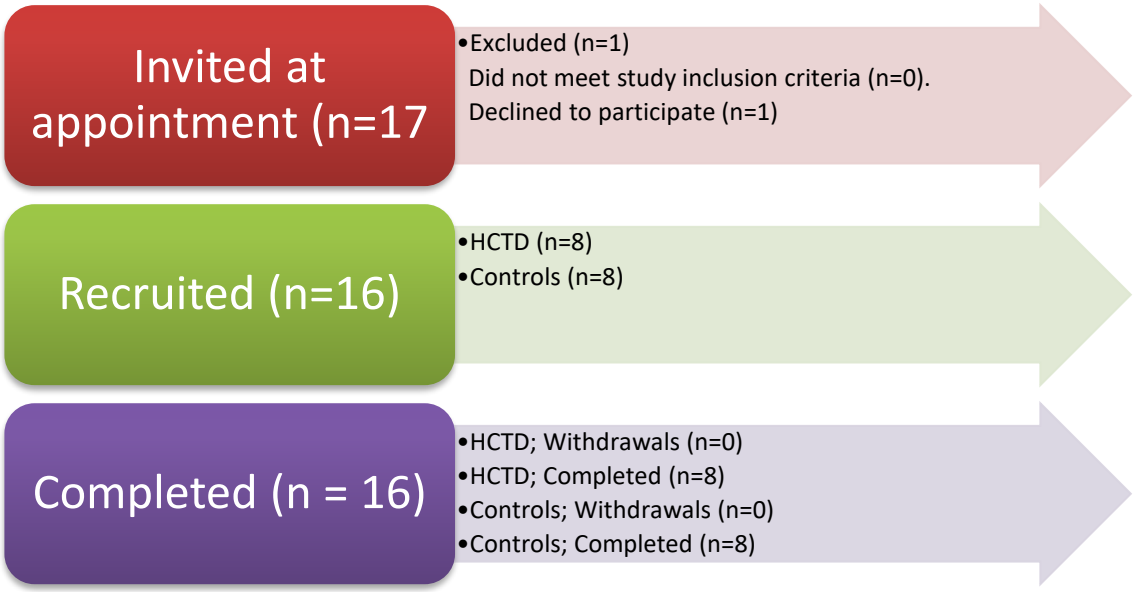


Fig. 59. *Chart demonstrating the recruitment and completion of participants for experiment 4.*

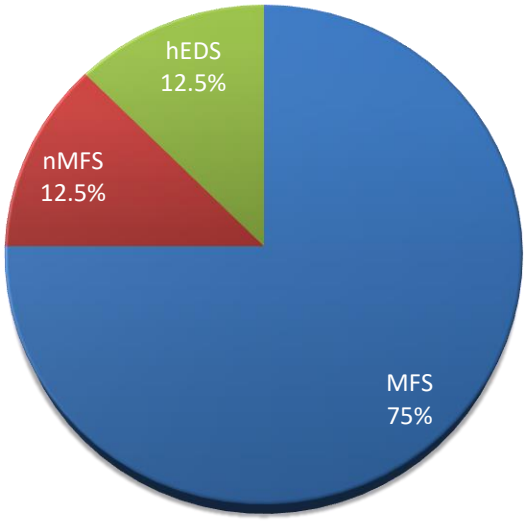


Fig. 60. *Baseline characteristics of connective tissue group (n = 8). Six participants had Marfan's Syndrome (MFS), 1 had characteristics that were similar to MFS and was diagnosed with non-Marfan's fibrillopathy (nMFS). One participant had hypermobile Ehlers Danlos Syndrome (hEDS).*

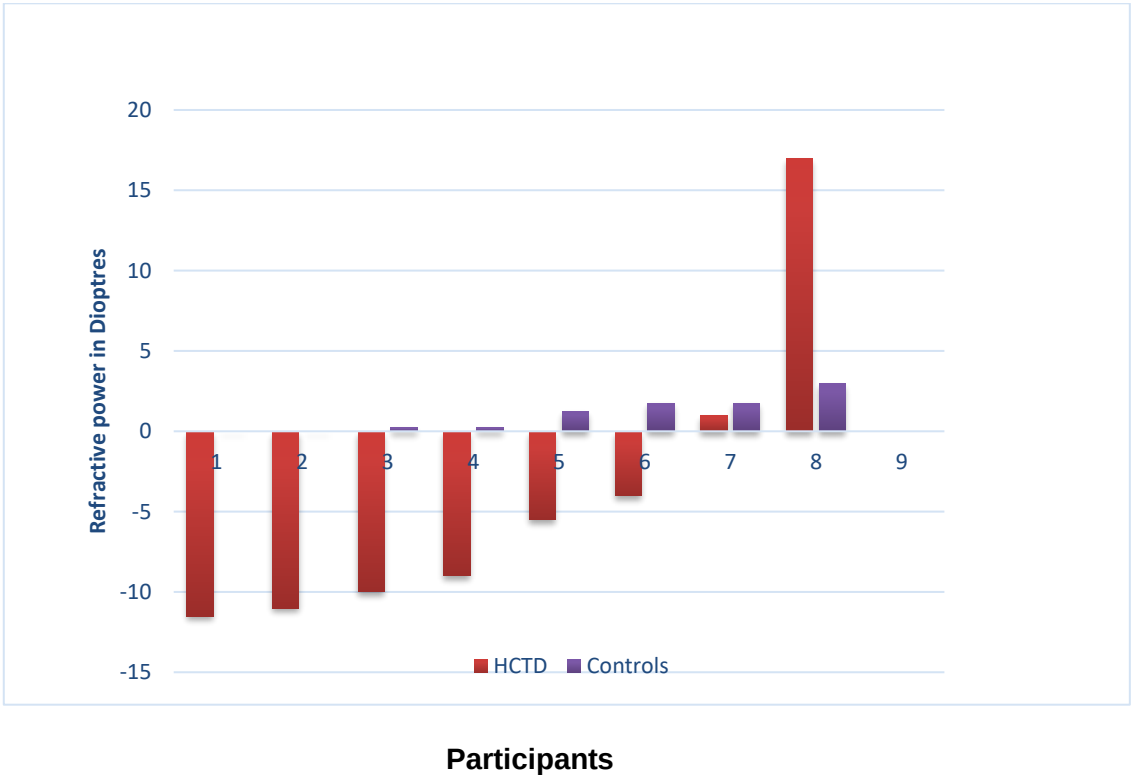


Fig. 61. *This graph demonstrates the wide range of high refractive defects found in the HCTD group compared with the low prescriptions found in the controls.*

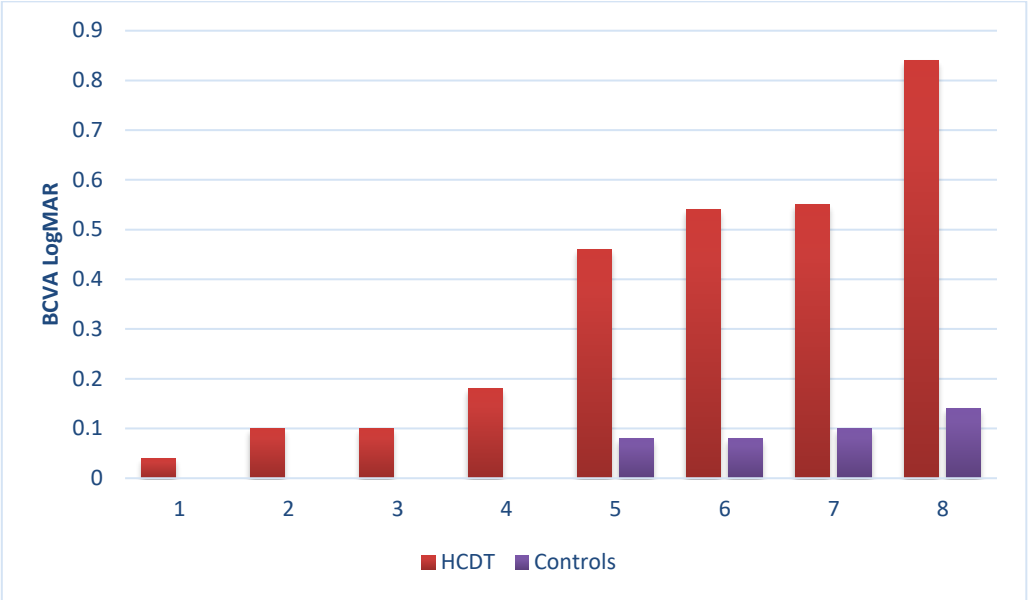


Fig. 62. *This graph of BCVA comparison demonstrates the wide-ranging poor BCVA of the HCDT cohort compared with the controls.*

The HCDT cohort comprised of children aged 5 to 15 years with a mean age of 8.25 (SD 3.3) years and a median of 7, five of which were female and 3 were male. The majority of the group were White (7), 1 was Asian. One had a peripheral corneal scar and the others had clear corneas. Two of the group had strabismus, none had nystagmus. Six had MFS, 1 had a non-Marfan's fibrinopathy (nMFS) and 1 had hypermobile EDS (hEDS). Five of the children with MFS had EL, one had phacodonesis, most were moderate to high myopes with some having high levels of astigmatism. These children had generally poor BCVA which ranged from + 0.10 to +0.84 LogMAR. One of the children with MFS also had glaucoma. The child with hEDS was a low hypermetrope with the best visual acuity out of the group (+0.04 LogMAR) and the child with nMFS had bilateral EL high myopia (-5.50 D) and poor BCVA (+0.46 LogMAR) (Table 20). Axial length was recorded for two of the children both of whom had MFS. The child with high myopia of -11.00 D had an axial length of 23.49 mm and the child with high astigmatism of -9.00 D had an axial length of 22.98 mm.

All RBT_{on} measurements were higher than GAT in the HCTD cohort and the majority of RBT_{off} values were lower than RBT_{on} with only one being equal.

The healthy controls were age matched as closely as possible to the HCTD cohort with an age range of 6 to 15 years and a mean of 9.4 (SD 3.2) and median of 9 years. Five were

female, 3 were male and none had strabismus, nystagmus, corneal defects or any ocular disease. Six were hyperopes with astigmatism of ≤ -2.00D and two were emmetropes. BCVA was generally good and ranged from 0.00 to +0.14 LogMAR.

In addition, the six eyes from children with MFS were compared with 6 age matched healthy controls. RBT_{off} measurements in the MFS cohort ranged from 14 to 20 mmHg with a mean of 17.3 (SD 2.1) mmHg and RBT_{on} ranged from 16 to 22 mmHg with a mean of 19.5 (SD 2.1) mmHg (Table 21). RBT_{off} measurements in the age matched healthy controls ranged from 10 to 22 mmHg with a mean of 16.8 (SD 4.5) mmHg and RBT_{on} ranged from 11 to 24 mmHg with a mean of 17.3 (SD 4.9) mmHg (Table 22). No significant interaction was found between the participant's condition and IOP, Wilks' Lambda = 0.91, F (1, 10) = .943, p = .354, partial η^2 = .086. There was no significant main effect for IOP, which suggests that the difference between RBT_{off} and RBT_{on} was not significantly affected by MFS, although mean RBT_{off} was lower than mean RBT_{on} in both groups and the MFS group showed a greater difference than the controls Wilks' Lambda = 0.81, F (1, 10) = 2.415, p = .151, partial η^2 = .195. Levene's Test of Equality of Error Variances: RBT_{off} p = .081 and RBT_{on} p = 0.82 (Fig. 63).

MFS	Minimum	Maximum	Mean	SD
RBToff	14.0	20.0	17.3	2.1
RBTon	16.0	22.0	19.5	2.1

Values are in mmHg

Table 21. *Descriptive statistics for the MFS cohort, RBT_{off} and RBT_{on} (n = 6).*

Controls	Minimum	Maximum	Mean	SD
RBToff	10.0	22.0	16.8	4.5
RBTon	11.0	24.0	17.3	4.9

Values are in mmHg

Table 22. *Descriptive statistics for age matched controls, RBT_{off} and RBT_{on} (n = 6).*

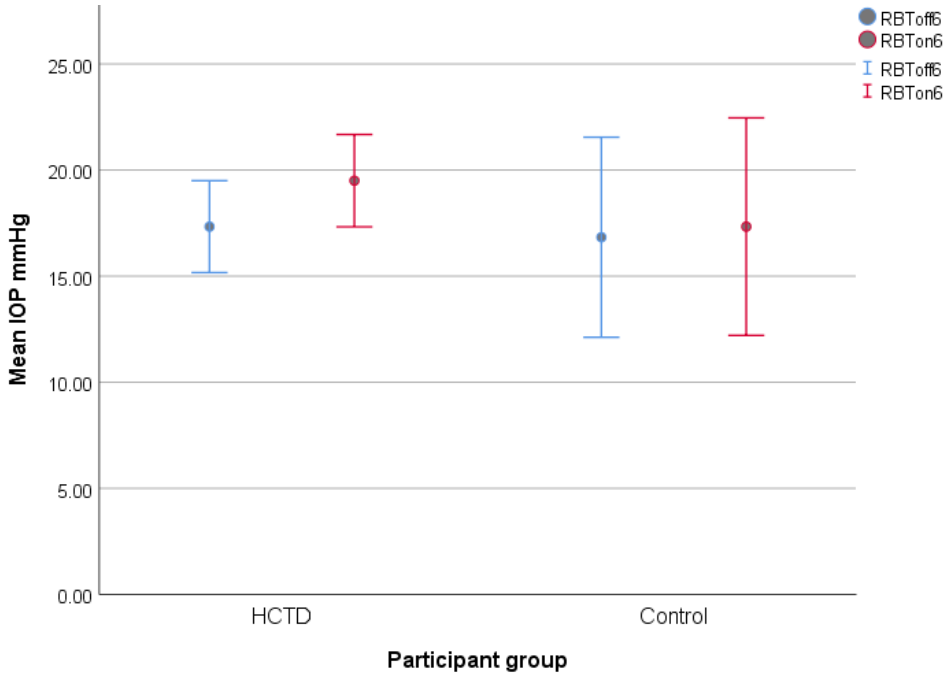


Fig. 63. *Graph showing the mean RBT_{off} and RBT_{on} IOPs for the 6 participants with MFS and age matched controls with 95% CI error bars. The graph demonstrates that although the difference was not significant, a greater mean difference was found between RBT_{off} and RBT_{on} in the MFS (HCTD) group.*

The seven eyes of children with MFS + nMFS were compared with 7 age matched healthy controls. RBT_{off} measurements in the MFS + nMFS cohort ranged from 10 to 20 mmHg with a mean of 16.3 (SD 3.4) mmHg and RBT_{on} ranged from 11 to 22 mmHg with a mean of 18.3 (SD 3.7) mmHg (Table 23). RBT_{off} measurements in the age matched healthy controls ranged from 10 to 22 mmHg with a mean of 16.4 (SD 4.2) mmHg and RBT_{on} ranged from 11 to 24 mmHg with a mean of 17.0 (SD 4.0) mmHg (Table. 24). No significant interaction was found between the participants condition and IOP, Wilks' Lambda = 0.93, F (1, 12) = .955, p = .348, partial η^2 = .074. There was no significant main effect for IOP, which suggests that the difference between RBT_{off} and RBT_{on} was not significantly affected by MFS, although mean RBT_{off} was lower than mean RBT_{on} in both groups and the MFS group showed a greater difference than the controls Wilks' Lambda = 0.80, F (1, 12) = 3.096, p = .104, partial η^2 = .205. Levene's Test of Equality of Error Variances: RBT_{off} p = 0.358 and RBT_{on} p = 0.608 (Fig. 64).

MFS+nMFS	Minimum	Maximum	Mean	SD
RBToff	10.0	20.0	16.3	3.4
RBTon	11.0	22.0	18.3	3.7

Values are in mmHg

Table 23. *Descriptive statistics for the MFS + nMFS cohort, RBT_{off} and RBT_{on} (n = 7).*

Controls	Minimum	Maximum	Mean	SD
RBToff	10.0	22.0	16.4	4.2
RBTon	11.0	24.0	17.0	4.0

Values are in mmHg

Table 24. *Descriptive statistics for age matched controls, RBT_{off} and RBT_{on} (n = 7).*

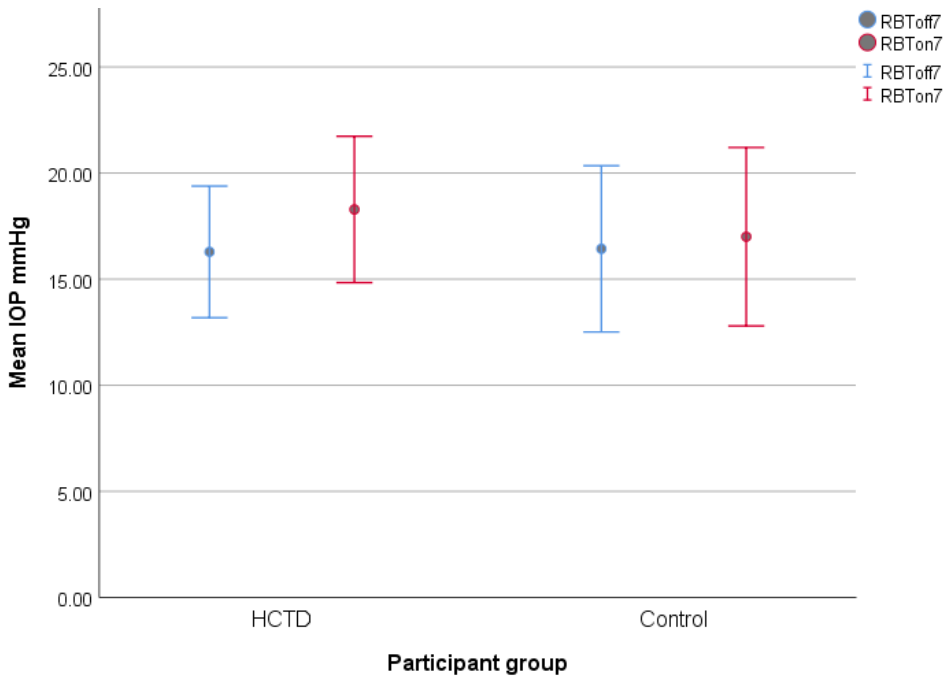


Fig. 64. *Graph showing the mean RBT_{off} and RBT_{on} IOPs for those with MFS + nMFS and age matched controls. The graph demonstrates that although the difference was not significant, there was a larger difference between RBT_{off} and RBT_{on} in the MFS+nMFS group.*

The mean differences between RBT_{on} and GAT from the eight eyes of children with HCTD were compared with 8 age matched healthy controls. RBT_{on} measurements in the HCTD cohort ranged from 11 to 22 mmHg with a mean of 18.1 (SD 3.5) mmHg and GAT ranged from 10 to 21 mmHg with a mean of 15.4 (SD 3.3) mmHg (Table 26). RBT_{on} measurements in

the age matched healthy controls ranged from 11 to 24 mmHg with a mean of 17.1 (SD 4.2) mmHg and GAT ranged from 10 to 20 mmHg with a mean of 15.3 (SD 3.4) mmHg (Table 26). No significant interaction was found between the participants condition and IOP, Wilks' Lambda = 0.97, F (1, 14) = .445, p = .516, partial η^2 = .031. However, there was a significant main effect for IOP, which suggests that the difference between RBT_{on} and GAT was significant in both groups with the HCTD group showing a greater difference than the controls Wilks' Lambda = 0.530, F (1, 14) = 12.429, p = .003, partial η^2 = .470. Levene's Test of Equality of Error Variances: RBT_{on} p = 0.637 and GAT p = 0.761 (Fig. 65).

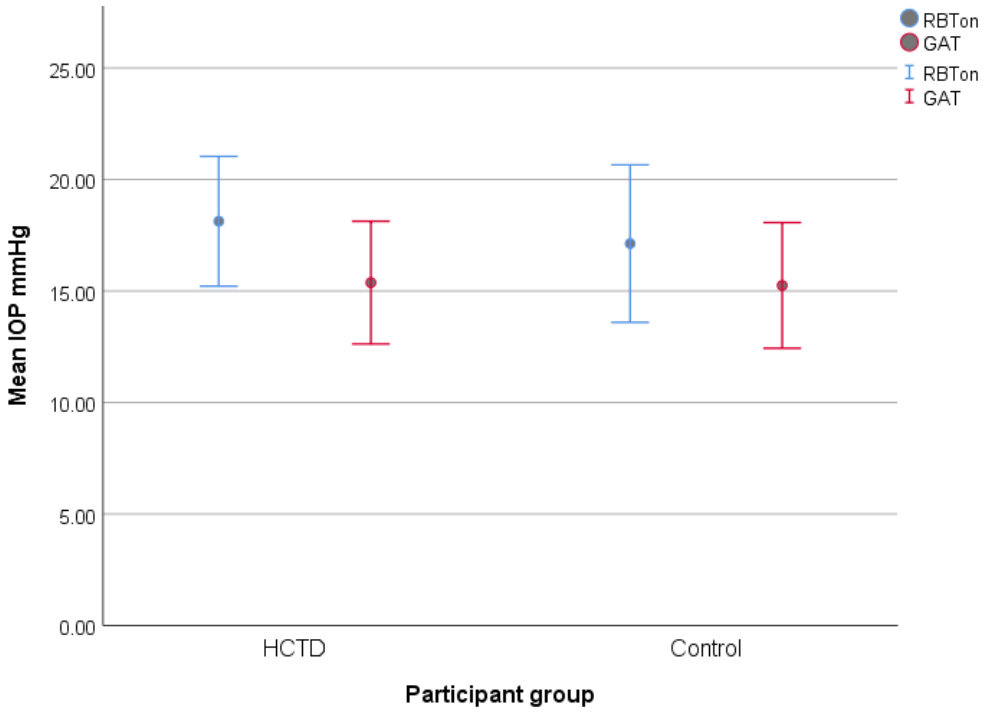


Fig. 65. *Graph showing RBT_{on} and GAT IOPs measured for those with HCTD and age matched controls with 95% CI error bars. This graph shows that mean RBT_{on} was significantly higher than GAT in both the HCTD* (2.7 (SD 2.1) mmHg p < .05) *and control cohorts* (1.9 (SD 3.1) mmHg p < .05)*, with the HCDT cohort having the greatest difference and the controls having a slightly larger standard deviation.*

HCTD	Minimum	Maximum	Mean	SD
RBT_{on}	11.0	22.0	18.1	3.5
GAT	10.0	21.0	15.4	3.3

Values are in mmHg

Table 25a. *Descriptive statistics for the whole HCTD cohort, RBT_{on} and GAT (n = 8).*

HCTD	Minimum	Maximum	Mean	SD
Sph	-9.00	+17.00	-1.13	9.0
Cyl	0.00	-9.00	-3.00	3.8

Values are in dioptres

Table 25b. *Descriptive statistics for refractive data HCTD cohort (n = 8).*

Controls	Minimum	Maximum	Mean	SD
RBT_{on}	11.0	24.0	17.1	4.2
GAT	10.0	20.0	15.3	3.4

Values are in mmHg

Table 26a. *Descriptive statistics for age matched controls, RBT_{on} and GAT (n = 8).*

HCTD	Minimum	Maximum	Mean	SD
Sph	0.00	+3.25	+1.47	1.28
Cyl	0.00	-2.00	-0.44	3.83

Values are in dioptres

Table 26b. *Descriptive statistics for refractive data control cohort (n = 8).*

4.1.4 Discussion

The main aim of Experiment 4 of this study was to examine the validity of suboptimal RBT_{off} measurements when compared with RBT_{on} readings in children with HCTDs and to compare the difference with healthy controls.

The Main finding from experiment 4 was that RBT_{off} was not significantly different from RBT_{on} in children with HCTDs and that the difference between RBT_{off} and RBT_{on} in these children was not significantly different from healthy controls. Therefore, there is evidence to suggest that suboptimal RBT measurements taken 3 mm temporal to the centre of the eye can be considered to be reliable and clinically useful. This is similar to results from previous studies in adults and children as discussed in section 3.2.3 (Yamashita *et al*., 2011; Beasley *et al*., 2013). It was noted that there was an insignificant mean difference, between RBT_{off} and RBT_{on} in the HCTD cohort than the controls. As previously mentioned in section 1.8.8.2, RBT is affected by hysteresis and corneal resistance factor which can be affected by HCTDs. Children with MFS can have low hysteresis, flatter corneas and high degrees of astigmatism (Kinori *et al*., 2017). Corneal curvature can affect RBT measurement with steeper curves leading to underestimation of IOP (Takenaka *et al*., (2011). The flatter corneal curve found in

MFS might be expected to lead to less underestimation of suboptimal RBT_{off} when compared with RBT_{on}. However, the opposite was found. One reason for this may be the presence of high astigmatism in some of the cohort with MFS, leading to steeper curves in some meridians (see Tables 25b and 26b for refractive data of HCTD and control cohorts). Chen *et al*., (2018) found that MFS patients had higher corneal astigmatism (total and anterior) than healthy controls. They also found that MFS patients with EL had flatter corneas and higher astigmatism than those without MFS. EL can give rise to high astigmatism with high myopia due to the dislocation of the lens which results in an optical axis through the periphery of the lens rather than the centre (Matsuo, 2015). Indeed, Table 25b illustrates the range of high spherical and astigmatic refractive errors found in the HCTD group. All of the MFS participants in this study including the child with nMFS had experienced EL. Three children with MFS had high astigmatism ranging from -6.00 to -9.00D. Only one child with MFS was aphakic due to lensectomy and was +17.00D with no astigmatism.

The child with nMFS had high myopia, congenital EL and poor visual acuity which are also found in MFS however, no systemic features were present. A diagnosis of nMFS was made by a consultant ophthalmologist with ongoing further investigation. Tsipouras *et al*., (1992) have proposed that simple dominant EL (SEL) is caused by mutations of FBN-1. Mutation of FBN-1 also gives rise to MFS as described previously in section 1.7.1. Mutation of different areas of the FBN-1 gene gives rise to different expressions of MFS with mutations of the end terminal C leading to mild phenotypes and mutations of the central region leading to severe disease (Comeglio *et al*., 2002). Fuchs and Rosenberg (1998) propose that SEL may be a different expression of MFS. For this reason, Comeglio *et al*., (2002) recommend that patients with SEL should have cardiac screening throughout their lives due to the late presentation of aortic dilation.

As axial length measurements were only available for two participants, it was not possible to conduct any useful analysis. However, it is interesting to note that the child age 8 years with MFS and high myopia of -11.00 D had an axial length of 23.49 mm which is similar to the results of a study by Maumenee (1981) who found and average of 24.65 (SD 2.21) mm in 160 patients with MFS and an age range of 3 to 59 years. By contrast, the child age 6 with MFS, hypermetropia of +5.00D and astigmatism of -9.00D had a shorter axial length of 22.98 mm.

As the HCTD group comprised of a mixture of diseases further analysis was undertaken using a smaller group of 6 MFS patients and a group of 7 participants consisting of MFS + nMFS to see if different results would be obtained. All the results were quite similar with no significant interaction found between the participant's condition and IOP and no significant main effect for IOP. However, in all three analyses mean RBT_{off} was lower than mean RBT_{on} with the HCTD group having the largest difference (Tables 18 to 24, Figs. 63 to 65)

The BCVA of the HCTD group was generally worse than the control group and the refractive defects were wide ranging and mostly myopic (Fig. 61 and 62). This agrees with Maumenee (1981) who found that 70.5% of the MFS cohort of 151 patients had a BCVA ≥ 20/40. Causes of reduced BCVA in MFS include amblyopia, retinal detachment, glaucoma, EL and cataract. Patients with MFS can have large corneal diameters, due to eye enlargement with diameters up to 13 mm (Maumanee, 1981). However, corneal diameter was not measured in this study. It would be interesting for future research to look at the association of corneal diameter and suboptimal RBT measurements.

No difference in corneal thickness between MFS patients and healthy controls (n = 160) was noted by Maumenee (1981). However, Sultan *et al*., (2002) used the Orbscan system to examine 60 eyes of patients with MFS and found an association between MFS and corneal thinning. They suggest that morphological changes of the FBN-1 microfibrils lead to stretching of the whole eye resulting in a thin flattened cornea at its centre and periphery. In addition, they used a confocal microscope to examine the corneas of participants with MFS and noted corneal thinning and an opaque stromal matrix with increased light scatter. As previously noted in section 1.7.1, children with EDS can also have thin corneas. However, this data was not available in the present study. RBT_{off} and RBT_{on} were very similar in the EDS participant with only a 1 mmHg difference and there were no co-existing ocular conditions.

CCT and corneal curvature can affect IOP measurement with thin flat corneas leading to underestimation of true IOP (Kotecha, 2007), (see sections1.6.3 and 1.8.7.2 of the present Thesis). Unfortunately, this can result in under diagnosis of chronic glaucoma in MFS patients (Sultan *et al*., 2002). In this study, no CCT or corneal curvature measurements were available from participants with MFS so it was not possible to compare results with previous studies.

Analysis of RBT_{on} and GAT data found that mean RBT_{on} was significantly higher than GAT in the both the HCTD (2.7 (SD 2.1) mmHg $p < .05$) and control cohorts (1.9 (SD 3.1) mmHg $p < .05$), with the HCDT group having the larger difference (Fig. 65). This agrees with previous studies on children with glaucoma by Dahlmann-Noor *et al*., 2013, and Flemmons *et al*., 2011b, and with the results of Experiment 1 of the present study as discussed in section 2.2.2. Kara *et al*., (2012) found that MFS patients have lower CH than healthy controls and MFS patients with EL have lower GAT IOP, CH and CRF than those without EL. They state that difference in corneal biomechanics between the HCTD group and the healthy controls may explain the larger difference between RBT_{on} and GAT in the HCTD group.

It is also interesting to note that the 95% CI limits for the controls was larger than the HCTDs, which indicates more variability in the control group measurements (Figs. 58, 63, 64, 65). Both the HCTDs and controls were experienced at having IOPs measured. However, the controls were slightly less experienced than the HCTDs because they did not visit the hospital as frequently and this may have contributed to this result.

Experiment 4 of this study was subject to several limitations including the small but powered sample size and inclusion of different types of HCTDs. Although the HCTD cohort was age matched as closely as possible with the controls it was not possible to gender match due to the difficulty in recruiting participants with rare diseases.

4.1.5 Conclusion

Children with HCTDs tend to have worse BCVA and higher spectacle prescriptions than healthy children. Suboptimal RBT_{off} measurements were not significantly affected by HCTD, although mean RBT_{off} were lower than optimal central RBT_{on} measurements in both the HCTD and control groups. The HCTD group demonstrated a greater difference between RBT_{off} and RBT_{on} measurements than the controls. However, the mean difference for the group was < 2 mmHg and is therefore not important clinically. This demonstrates the reliability of suboptimal RBT measurements in children with HCTDs for routine purposes however critical treatment decisions should not be solely based on these measurements. RBT_{on} was found to be statistically significantly higher than GAT in children with HCTDs and in healthy controls, with larger differences found in HCTD. However, both differences were less than 3mmHg, so this

is not clinically significant. Therefore, this study recommends that GAT measurements can be substituted by RBT_{on} in children with HCTDs except in critical cases.

Dissemination

The results of chapter 4 were presented at two prestigious conferences: UKEGS (2018) and UKPGS (2019) and at a BWCH audit (2019).

Chapter 5

Summary

5.1 Overall conclusion

Over the past 14 years, RBT has become has become a popular method of screening IOP in children in the Hospital Eye Service and in optometric practice as it is a child friendly device; however, there is a paucity of information concerning the agreement between RBT and GAT in children with glaucoma (Dahlmann-Noor *et al*., 2013). So far as the author is aware, not much is known about the reliability of suboptimal RBT measurements in children with glaucoma and HCTDs who may present with a range of co-existing characteristics. Therefore, the present study evolved from a clinical need to know more about the reliability of RBT in children with glaucoma and HCTDs.

The three main objectives of the present study were to examine the following:

1. The validity of RBT measurements in children with glaucoma (Experiment 1).
2. The reliability of suboptimal RBT readings in children with glaucoma and the association of their co-existing conditions (Experiments 2 & 3).
3. The reliability of suboptimal RBT measurements in children with HCTDs (Experiment 4).

Thirty-four children with glaucoma from the Eye Department of BWCH were recruited for objectives 1 and 2. Eight children with HCTD and 8 controls were recruited from the same Eye Department for objective 3. Following consent/assent, all had RBT_{on}, RBT_{off} and GAT IOPs measured. In addition, co-existing characteristics were noted.

IOP status	Group 1	Group 2
A	-5 to 2 mmHg	3 to 11 mmHg
B	≤ 3 mmHg	> 3 mmHg
C	< 3 mmHg	≥ 3 mmHg

Table 27. *IOP status groups: Experiment 1 used IOP statuses A and B. Experiment 3 used IOP status C.*

Experiment 1 found that RBT_{on} significantly overestimated GAT by an average of 2.4 (SD 3.0) mmHg, $p < 0.000$, with 95% limits of agreement of -3.8 to 8.28 mmHg, when compared with GAT in children with glaucoma. This is similar to the results of a paediatric study by

Flemmons *et al*., 2011b (2.3, SD 3.7 mmHg, $p < 0.0001$). Dahlmann-Noor *et al*., (2013) also found that RBT overestimated GAT significantly especially at high IOPs in children with glaucoma. Although RBT_{on} overestimated GAT by a statistically significant amount in the present study, the difference was less important clinically, where differences of < 3 mmHg are acceptable (Flemmons *et al*., 2011b, Dahlmann-Noor *et al*., 2013).

The present study found a significant positive correlation ($r = .919$, $n = 34$, $p < 0.01$) between RBT_{on} and GAT, which agrees with previous studies in adults and children. However, Dahlmann-Noor *et al*., (2013) did not find a good agreement between RBT and GAT in their study and postulate that this may be due to confounding co-existing corneal conditions i.e. multiple surgeries and corneal oedema. They point out that Flemmons *et al*., (2011b) repeated RBT measurements until minimal variability was obtained, eliminating measurements that were taken when the patient was nervous or squeezing the eyelids. The present study did not repeat measurements and was subject to co-existing conditions which will be discussed further on.

RBT_{on} was within 3 mmHg of GAT in 62% of participants which compares favourably with previous studies by Flemmons *et al*., (2011b) and Dahlmann-Noor *et al*., (2013). However, in adult studies a higher percentage has been found (Fernandez et al. 2005; Beasley et al. 2013; Iliev et al. 2006; Gao et al. 2017) possibly because adults cooperate better than children when having their IOPs measured. It is interesting to note that approximately one third of RBT_{on} readings were > 3 mmHg GAT which is important clinically. Therefore, it is recommended by the author and by previous studies that a second instrument is used to verify IOPs when making critical clinical decisions (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013). The study by Flemmons *et al*., (2011b) found no association between age and RBT measurements that were higher than GAT. However, the present study did find an association between age of the child and IOP status A. IOP status A was divided into two groups (group 1, -5 to 2 mmHg and group 2, 3 to 11 mmHg), The present study found that approximately one fifth of RBT_{off} measurements were lower than GAT in children aged 9 years and under and 90% of RBT_{on} measurements were higher than GAT in children over 9. The present study looked at the range of IOP differences rather than the “less than” or “more than” values of IOP status B (group 1 ≤ 3 mmHg and group 2 > 3 mmHg) used by Flemmons *et al*.,

(2011b). In fact, the present study also examined the association between age and IOP status B and like Flemmons *et al*., (2011b) did not find any association. Similar studies in children and adults have found that RBT_{on} overestimates GAT with the difference increasing with higher IOPs (i.e ranging from > 20 to 28 mmHg, depending on the study) (Fernandez *et al*., 2005; Flemmons *et al*., 2011b; Beasley *et al*., 2013; Dahlmann-Noor *et al*., 2013; Grigorian *et al*., 2015; Iliev *et al*., 2006). However, results have varied with larger mean differences found where the Icare device is fixed to the slit lamp giving rise to more perpendicular measurements (Muttuvellu *et al*., 2012). Most studies have used a freely held device as this is how it is used in clinical practice, which may have resulted in angular measurements which can lead to lower IOP values. Indeed Beasley *et al*., (2013) found that RBT readings taken at an angle 10 degrees from the normal straight position were statistically significantly different from RBT measurements when used in the straight position. However, as explained in section 3.3.4, the difference was < 1mmHg so was not clinically significant. In addition, younger children can be uncooperative, leading to less accurate measurements (Dahlmann-Noor *et al*., 2013). However, the children in the present study were accustomed to having their IOPs measured and RBT has been shown to be well tolerated by children (Sahin *et al*., 2007). The present study also found that IOP (RBT_{on}, RBT_{off} and GAT) in children rises until the age of 7 (see Figs. 35, 36, and 46), following which it decreases and starts to rise again from the age of 10 years. This agrees somewhat with Eisenberg *et al*., (1998) who found that IOP in children rises linearly until the age of 10.

True IOP is measured by manometry (Kniestedt *et al*., 2008). However, GAT is the gold standard (Munkwitz *et al*., 2008). GAT is heavily influenced by corneal mechanics and CCT, which can lead to misclassification of pressure-related diseases (Medeiros and Weinreb, 2006). As young children tend to have thinner corneas one would think that GAT would overestimate their IOP. However, Eisenburg *et al*., (1998) found that GAT underestimated manometry in young children. As the present study found that one fifth of RBT_{on} was lower than GAT in children under 9 years, it may be even further away from the real IOP as measured by manometry. Therefore, the present study also suggests that GAT in children is really only comparable with adults from the age of 10 or11 when corneal thickness reaches adult levels (Eisenberg *et al*., 1998). The present study also recommends that RBT is not a

good substitute for GAT in children aged 9 and under in critical cases and a second tonometer should be used to verify RBT and GAT measurements.

The results from Experiment 2 found that mean RBT_{off} was slightly lower but not significantly different to mean RBT_{on}. (-0.71, (SD 2.7) mmHg). Various theories have been proposed by previous authors for this, from the greater angle at which the RBT probe hits the cornea for RBT_{off}, to the peripheral cornea being thicker and softer than the central cornea, both producing lower values (Takenaka *et al*., 2011, Boote *et al*., 2003).

There was a good correlation between RBT_{off} in the temporal cornea and RBT_{on} (0.933, $p < 0.01$), between 9 and 48 mmHg which is useful to know clinically. RBT_{off} and RBT_{on} differed by 1 mmHg or less in 41.2 % of children with glaucoma in the present study compared with 73.2% in healthy adults from a previous study by Queiros *et al*., (2007). Therefore, it is possible that RBT_{off} may be less reliable in children with glaucoma, which may be due to lack of cooperation during IOP measurement. Two thirds of ($RBT_{off} – RBT_{on}$) were < 3 mmHg in the present study. However, one third of differences were equal or greater and this has implications for clinical decisions where small changes matter.

Experiment 2 results also demonstrated that peripheral RBT_{off} values were significantly different (1.64 (SD 3.5) mmHg) but closer to GAT than RBT_{on} (2.4 (SD 3.0) mmHg). It was noted that RBT_{on} was often higher than GAT and RBT_{off} was often lower than RBT_{on}. The correlation between RBT_{off} and GAT was reasonable ($r = 0.66$, $p < 0.01$) but not as strong as between RBT_{on} and GAT ($r = 0.919$, $p < 0.01$) especially at higher IOPs above 30 mmHg.

Experiment 3 examined the association of co-existing characteristics (see Table 16) and IOP status C in children with glaucoma. IOP status C consisted of two groups of (RBT_{off}-RBT_{on}), group 1; < 3 mmHg and group 2; ≥ 3 mmHg.

Age was not significantly associated with the difference between RBT_{off} and $RBT_{on.}$ (IOP status C). However, it was noted that the difference was larger in children above 8 years old. It is interesting to note that in the present study at around the age of 8 or 9 years both the relationships between RBT_{on} and GAT and between RBT_{off} and RBT_{on} changed and around the age of 10 CCT increases to adult levels (Eisenberg *et al*., (1998).

The difference between RBT_{off} and RBT_{on} was not significantly associated with gender. However, RBT_{off} was closer to RBT_{on} more often in male children in this experiment

(Fig. 47). This may be due to the flatter corneas found in male children (Yamashita. 2011). Only a small difference between the mean IOP (RBT_{on}) in males and females was found, with males having a slightly higher mean (20 (SD 10.6) mmHg) than females (19.5 (SD 6.2) mmHg). In addition, mean IOP GAT values were analysed and it was found that mean IOP (GAT) in males was also higher than females and the difference between the genders was greater (males: mean GAT 17.9 (SD 8.2) mmHg, females: mean GAT16.6 (SD 5.6) mmHg). Previous studies in adults have found the reverse (Leske *et al*., 1997; Hashemi *et al*., 2018; Xu *et al*., 2005; Memarzadeh *et al*., 2008). However, these studies used only used GAT to measure IOP and the mean differences in the present study were small.

It was expected that nystagmus or strabismus may lead to larger differences between RBT_{on} and RBT_{off}, due to the movement of the eye and increased angle of RBT_{off} measurements. However, no association was found. Children with nystagmus can adopt a head posture to find the null point in order to reduce eye movements (Casteels *et al*.,1992), which may explain this. The present study demonstrates the high prevalence of strabismus in children with glaucoma (50%). This compares with approximately 3% in a healthy multi-ethnic paediatric population (McKean-Cowdin, 2013).

Refractive error was not associated with the difference between RBT_{off} and $RBT_{on.,}$ even though myopes have flatter corneas and lower CH than hyperopes (Chui *et al*., 2008; Yamashita *et al*., 2011). However, the difference between RBT_{off} and $RBT_{on.,}$ was < 3 mmHg more often in the high refractive error group than the lower, which was surprising. The reason for this was unclear. BCVA was also not associated with difference between RBT_{off} and $RBT_{on,}$ however the group with the best BCVA did have a higher number of measurements that were < 3mmHg different which was expected because they had better fixation.

General health was not associated with the difference between RBT_{off} and $RBT_{on..}$ However, the healthy group had a higher number of measurements that were < 3 mmHg different which was not surprising as these participants were more able to cooperate. Several general health conditions, that are known to occur with childhood glaucoma were identified from the cohort. JIA, which is associated with uveitic glaucoma (Kanski, 1990) and congenital heart defects, which are associated with ARS and ASD (Idrees *et al.*, 2006). However, 50% of the cohort did not have any general health problems.

The type of glaucoma was not associated with IOP status C. As can be seen in Table 16, the types of glaucoma were divided into five groups. Aphakic glaucoma following cataract surgery was the most common cause of glaucoma in the participants of the present study, followed by "others", PCG, uveitic and JOAG. The "others" group included glaucoma due to syndromes such as Peter's anomaly and ARS.

RBT_{off} - RBT_{on} was ≥ 3 mmHg in the majority of the uveitic group. This may be explained by the relatively poor vision and fixation in these participants which has been discussed in section 3.3.4 under BCVA.

The main finding from Experiment 4 was that no significant interaction was found between the HDTD group and IOP (Wilks' Lambda = 95, F (1,14) = .767, p = .396, partial η^2 = .052). There was also no significant main effect for IOP, which suggests that the difference between RBT_{off} and RBT_{on} was not significantly affected by HCTD. Therefore, temporal RBT_{off} measurements were found to be clinically useful in children with HCTDs. Interestingly, although the difference was not significant, the HCTD group did show a larger difference between RBT_{off} and RBT_{on} than the control group (Fig. 58). Children with MFS have low hysteresis, flatter corneas and high astigmatism (Kinori *et al*., 2017). High astigmatism is associated with EL (Chen *et al*., (2018) and 7 out of the 8 participants in the HCTD group had experienced EL. RBT is affected by CH, CRF and corneal curvature (Chui *et al*, 2008) which may explain the larger difference between RBT_{off} and RBT_{on} in the HCTD group.

As the HCTD group consisted of 6 participants with MFS, one with nMFS and one with hEDS, further analysis was conducted to see whether removing the non-MFS HCTDs would have any influence on results. However, no significant interaction between the MFS group (without the hEDS and nMFS) and IOP was found. In addition, no interaction between the MFS + nMFS group (without hEDS) and IOP was found. As GAT measurements were also taken, it was possible to compare RBT_{on} and GAT within and between the HCTD and control groups. No significant interaction was found between the participant's condition (either HCTD or control) and IOP. However, as expected a significant main effect was found between RBT_{on} and GAT. This result was not unexpected and agrees with previous results from Experiment 1 of this study. It was also interesting to note that a greater mean difference was found between RBT_{on} and GAT in the HCTD group than in the controls (Fig. 65), which may be a result of the

differences in CH, CRF and astigmatism between the HCTD and control groups as mentioned above.

The characteristics of the HCTD group are listed in Table 20. It is interesting to note that all of the children with MFS and nMFS had high spectacle prescriptions ranging from +17.00 D to -11.00 D with high astigmatism of up to -9.00 D. In general, visual acuity was poor in the MFS participants ranging from 0.10 to 0.84 LogMAR. This agrees with the findings by Maumenee (1981) that patients with MFS tend to have high myopia and poor BCVA. By contrast the controls had good vision and low refractive errors (Figs. 61 & 62).

However, in spite of all the differences between the HCDT group and the controls, the present study has shown that RBT measurements taken 3 mm temporal to the centre of the cornea in children with HCTDs are reliable and clinically useful.

5.2 Study limitations

The present study was subject to the following limitations;

RBT was compared with GAT with no independent reference to decide which method was closer to the true IOP.

It was not possible to undertake blind measurements due to the busy clinical setting so bias may have been introduced. Iliev *et al*., (2006) were also unable to undertake blind measurements for the same reason.

In the present study measurements were taken by two observers, both of whom were highly experienced at using GAT and RBT. Ninety percent of all RBT and GAT measurements were taken by one observer. However, the mean and SD of (RBT_{on} – GAT) were similar to results from previous studies by Flemmons *et al*., (2011b) and Dahlmann-Noor *et al*., (2013), where the experiments were blinded and intra/inter observer variability showed no bias, which helps to validate the results from the present study.

Randomization was limited to choosing the right or left eye and the order of RBT_{off} and RBT_{on}. In line with other studies, it was not possible to randomize RBT and GAT measurements due to the topical anaesthetic used for GAT and the displacement of fluid by GAT. RBT measurements had to be taken first (Fernandez *et al*., 2005; Iliev *et al*., 2006; Lopez-Cabalero 2007; Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013).

The sample size for the present study was powered but was small out of necessity due to the rarity of paediatric glaucoma and HCTDs and the practicality of collecting data in a clinical setting within a reasonable length of time. The sample size needed was calculated using the G*Power 3.1 power analysis program which uses values chosen by the operator in order to achieve the required statistical power, significance level and population effect size (small, medium, large), (Faul *et al*., 2009). The statistical power indicates the probability that a true effect will be detected, with a low power leading to unreliable conclusions (Faul *et al*., 2009). A power of 80%, a medium effect size with and an alpha level of 0.05 was selected when calculating the sample size for the present study. These values were selected after consultation with statistician Dr Richard Armstrong of Aston University.

Enough data was collected to fulfil the required sample sizes for all of the experiments. Unfortunately, it was not possible to collect enough data concerning corneal thickness, axial length and corneal curvature due to lack of availability in the participants' notes.

The co-existing characteristics in table 16 were not associated with (RBT_{off} - RBT_{on}). in children with glaucoma. However, some of the results were not valid as larger sample sizes were needed for Chi square calculations. Future studies with larger sample sizes are needed to look at the association of type of treatment, type of glaucoma and ethnicity with (RBT_{off} - RBT_{on}).

A confounding factor in this study was that the children with glaucoma had different types of glaucoma and had received different treatments from surgery to medication or both and any or all of this may have affected the IOP measurement (Sun *et al*., 2009; Agarwald *et al*., 2012). Further more detailed research is needed regarding the effect of type of glaucoma and treatment on IOP measurements.

One half of all participants were recorded as having strabismus. However, the present research study was pseudorandom and the strabismic eye was not always chosen to be the study eye. This may have been a confounding factor when looking at the reliability of suboptimal RBT in these children.

A further limitation was that many of the participants were young children who may therefore, move or squeeze their eyelids on IOP measurement. However, fortunately the children in the

present study were accustomed to having their IOPs measured by both RBT and GAT, so were less likely to do so.

Although a trial frame was placed on the child's face to guide the position of the RBT it was not possible to be sure that the measurements were taken right at the centre of the cornea, 3 mm temporally and at the correct distance from the cornea, due to inaccuracy with alignment. However, this does reflect how the instrument is used clinically.

5.3 Further research recommendations

As far as the author is aware there are few studies that examine the validity of RBT measurements in children with glaucoma when compared with GAT (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013). Grigorian *et al*. (2015) used a mixed paediatric cohort, 13% of whom had glaucoma. Martinez-de-la-Casa *et al*. (2009) and Borrego Sanz *et al*. (2016) compared RBT with hand-held versions of GAT. By contrast, there are several studies in adults with and without glaucoma that have compared RBT with GAT (Fernandes *et al*., 2005; Brusini *et al*., 2006; Davies *et al*., 2006; Munkvitz *et al*., 2008; Poostchi *et al*., 2009; Beasley *et al*., 2013; Kim *et al*., 2013). In addition, to date most paediatric studies comparing RBT with GAT have had small sample sizes of 70 to 214 participants (Flemmons *et al*., 2011b; Dahlmann-Noor *et al*., 2013; Grigorian *et al*., 2015). Although a reasonable power of 80% was achieved when calculating the sample size, a higher power would have resulted in a larger sample size and even stronger results. However, due to the difficulty in participant recruitment this would have been unrealistic and difficult to obtain given the time constraint for data collection. More studies are needed to examine the reliability of RBT in children with glaucoma and the author recommends that future studies enrol more participants if possible. To date there is a paucity of information regarding the association of children's age with RBT measurement. As previous research by Flemmons *et al*. (2011b) had not found an association between the child's age and the difference between RBT and GAT measurements, the result from the present study was unexpected. So far as the author is aware the present study is the first to divide the difference between RBT_{on} and RBT_{off} using the range of differences in the way it did. Therefore, further research comparing RBT with GAT using this method is recommended for children with glaucoma.

As previously discussed in section 3.2.4, most studies have not used a fixed RBT device to measure IOP, which may have resulted in underestimation of IOP. Therefore, future studies should consider using a fixed device attached to a slit lamp to enable more precision.

Most but not all RBT_{off} measurements were lower than RBT_{on}, and the exact reason why is unclear. As far as the author is aware this is the first study to examine suboptimal RBT measurements in children with glaucoma. As there is a clinical need to know more about the validity of suboptimal RBT measurements, further research in this area is needed.

As far as the author is aware the present study is the first study to examine the association off co-existing characteristics (Fig. 16) and suboptimal RBT measurement in children with glaucoma. Although only an association with age was found, the results produced interesting graphs (section 3.3.3) and further larger studies are needed to examine these the association of these characteristics with RBT in more detail.

Temporal corneal suboptimal RBT measurements were chosen by the present study because from them the authors experience this is a less invasive position than central measurement in children. Previous studies have indicated that this may be a good alternative to central RBT measurements in diseased and scarred corneas of adults (Chui *et al*., 2008; Yamashita *et al*., 2011) and the present study supports this in children. However, comparisons were not made with other areas of the cornea and future studies are needed to examine this.

The present study examined the validity of RBT measurements 3 mm temporal to the geometric optical centre of the cornea. Further research is recommended to examine how far out on the cornea RBT measurements can be taken whilst still being reliable and clinically useful.

The results of Experiment 4 show the larger but not significant difference between RBT_{off} and RBT_{on} in the HCTD group. This was interesting and is not completely understood. However, it may be due to the poor vision in the HCTD participants, leading to poor fixation, or the difference in corneal biomechanics between them and the controls. In addition, as far as the author is aware, the present study is the first to examine this and the sample size although powered was small. Therefore, further research on IOP measurement in children with HCTDs is needed.

5.4 Clinical recommendations:

1. RBT measurements in children with glaucoma differ from GAT but are useful clinically.
2. The association between RBT and GAT varies with the age of the child, with up to one fifth of RBT measurements lower than GAT in children under 10 years.
3. Suboptimal temporal RBT measurements taken 3 mm from the geometric centre of the cornea are reliable in children with glaucoma with a range of co-existing conditions and in children with HCTD.
4. RBT is a useful method of estimating IOP in children. However, a second method should be used to corroborate findings when making critical decisions.
5. As far as the author is aware, currently the College of Optometrists Clinical Management Guidelines (CMG's) do not have any guidance concerning children's glaucoma and HCTDs (College of Optometrists, accessed 29/11/2019). Therefore, the present study recommends that this is addressed because from the author's experience there is a paucity of knowledge and awareness about these conditions amongst fellow optometrists.

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Appendix 1.0. Abstracts, summaries, conference presentations.

Presentations:

A pre-study power point presentation entitled “Limitations and practical tips for rebound Icare tonometry” was presented by Nicola Sabokbar at a pediatric ophthalmology regional trainees teaching session at Birmingham and Midland Eye Centre (BMEC) on the 7th October 2015. This was at the invitation of consultant ophthalmologist Mr Joseph Abbott of BCH.

The present study was registered with the International Standard Randomized Controlled Trials Number (ISRCTN) registry which is an international clinical trial registry recognized by the World health Organization. A summary of the results was added to the registry when data collection ended and the study was closed (see: http://www.isrctn.com/ISRCTN15954407. BasicResults_3Mar19.pdf)

The results of the present study were presented at three prestigious conferences:

1) Oral presentation at UKISCRS (2017)
2) A poster was presented at UKEGS (2018)
3) Oral presentation at UKPGS (2019).

In addition, in June 2019, the results were presented at an Eye Department audit at BWCH.

Abstract presented at UKPGS 2019

Abstract

The validity and reliability of intraocular pressure measurement, using rebound tonometry in young children with ocular and systemic disease

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Aims:
To study the validity of rebound tonometry (RBT) and the reliability of off-axis intraocular pressure (IOP) measurements in children with glaucoma and heritable connective tissue disease (HCTD)

Methods:

RBT measurements were taken at the centre of the cornea (RBT_{on}) and 3 mmHg temporally (RBT_{off}) on one eye of 50 children (34 glaucoma, 8 HCDT, 8 healthy controls) followed by Goldmann applanation tonometry (GAT). The agreement of RBT_{on} with GAT and RBT_{off} with RBT_{on} was assessed. The association with age and other co-existing factors was also considered. RBT_{off} and RBT_{on} measurements in children with HCTD and controls were compared using a mixed factor ANOVA.

Results:

A high correlation was found between RBT_{on} and GAT ($r = 0.961$; $p = 0.000$) and between RBT_{off} and RBT_{on} ($r = 0.960$; $p = 0.000$). RBT_{on} was significantly higher than GAT (2.4, SD3.0 mmHg). No significant difference ($p = .10$) was found between RBT_{off} and RBT_{on} (-0.7, SD 2.7mmHg). A significant association was found between age and IOP; RBT_{on} was on average lower than GAT in younger children (< 9 years) and higher in older children.
RBT_{off} and RBT_{on} were not significantly affected by HCDT, although mean RBT_{off} was lower than mean RBT_{on} in both groups and the connective tissue group showed a greater difference than the controls Wilks' Lambda ($p=0.060$).

Conclusion: Differences between RBT_{on} and GAT were statistically, but not clinically, significant. RBT_{off} measurements are reliable in children with glaucoma and HCTDs and can be closer to GAT than RBT_{on}. On average, RBT_{on} measurements underestimate GAT in younger children and overestimate in older children.

Appendix 2.0: Raw data used for Experiments 1, 2 and 3

ID	1	2	3	4	5	6	7	8	9	10	11
GAT	13	12	29	20	12	16	9	12	16	13	24
RBToff	12	9	29	20	14	13	10	15	16	15	29
RBTon	15	12	30	24	15	15	12	15	19	16	28
Corn def	yes	yes	yes	no	no	yes	yes	yes	no	no	yes
Nystag	no	yes	no	no	no	no	yes	no	no	no	yes
Health	poor	good	poor	poor	poor	poor	good	good	poor	good	poor
Gender	female	male	female	female	male	male	female	male	female	male	female
Ethnicity	White	White	White	White	Asian	Asian	White	White	White	White	White
Treat-ment	Both	Both	Medical	Medical	Both	Both	Both	Both	Both	Both	Both
BCVA	0.58	0.6	0.1	0	0.2	0.12	0.48	0	-0.16	0.84	0.28
Strabis-mus	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	Yes
Oc hist	PCG	Aphakic	JOAG	Sturge Weber	Uveitic	Micropht halmia	Aphakic	PCG	Uveitic	Aphakic	Aphakic
Rx	-11.00/-2.00 x 20	+16.75/+1.75 x 135	-0.50/-0.50 x 87	+1.00	High +ve > 6.00	High +ve > 6.00	+18.00	+2.25/-2.00 x 180	0.00	+12.00	+21.00
Age	6	4	6	11	12	15	11	5	15	6	15
Eye	Right	Left	Right	Right	Left	Right	Right	Left	Right	Right	Right
CC (mm)	-	-	-	-	-	-	7.80 (180)	-	-	-	-
CCT (µm)	-	-	-	569	-	-	-	606	-	-	-
AL (mm)	-	-	-	-	-	-	-	-	-	-	-

24	23	22	21	20	19	18	17	16	15	14	13	12
10	13	16	12	23	10	37	16	30	15	15	22	12
9	9	17	14	30	10	52	17	32	15	18	27	13
11	11	13	16	27	9	48	17	34	10	18	29	17
yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	yes
no	no	no	no	yes	no	no	yes	no	yes	yes	yes	no
good	poor	good	good	poor	poor	good	good	good	good	poor	good	good
male	male	male	male	male	male	male	male	male	female	female	female	female
White	White	White	White	Asian	White	White	Mixed	White	Asian	Asian	White	White
Both	Both	Surgical	Medical	Both	Both	Both	Both	Both	Medical	Both	Both	Both
1.15	0.96	0.14	0	1.3	0	1.6	0.08	0.14	0.8	0.85	0.4	0.38
No	No	Yes	No	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes
PCG	Axenfeld Rieger	Aphakic	JOAG	Aphakic	PCG	Traumatic	Aphakic	PCG	Aphakic	Aphakic	Aphakic	Aphakic
-10.00	-1.50/-4.50 x 105	+7.00/-1.75 x 135	+0.75/-0.50 x 140	+13.00	-2.00/-0.50 x 140	0.00	+1.75/-2.25 x 177.5	+0.75/-3.00 x 70	+17.00	+18.00	+14.00/-1.50 x 180	+9.50
6	8	10	11	6	6	14	6	7	9	11	13	5
Right	Left	Left	Right	Left	Left	Right	Left	Left	Left	Right	Right	Right
-	-	-	-	-	-	-	7.54 (180)	-	-	-	-	-
-	-	-	549	-	-	-	-	-	-	834	-	-
-	-	-	-	-	-	23.70	16.70	21.70	-	-	-	-

34	33	32	31	30	29	28	27	26	25
19	19	12	18	15	15	20	38	18	18
20	24	12	20	18	16	25	39	14	22
20	23	13	19	17	20	24	45	18	19
yes	no	yes	yes	yes	yes	yes	yes	yes	no
no	no	yes	no	no	yes	yes	no	no	no
good	good	poor	poor	good	poor	good	poor	poor	good
feamle	male	female	male	female	male	female	male	male	male
Asian	White	White	White	Asian	Asian	Asian	Asian	Asian	Asian
Surgical	Medical	Medical	Both	Both	Both	Both	Medical	Medical	Medical
0.2	0.2	0.92	0.36	1.04	0.68	0.88	1.8	0	0.06
No	No	Yes	No	No	Yes	No	Yes	No	No
PCG	Aphakic	Aphakic	PCG	Peters anomaly	Micropht halmia	Micropht halmia	Uveitic	Uveitic	JOAG
+1.50/-1.75 x 15	12.50/-1.25 x 180	+12.00/-6.00 x45	-050/-0.50 x 55	-13.00/-0.75 x 29	-20.00	-2.50/-2.50 x 80	-4.00	+10.50/-1.00x 165	-3.00
7	7	13	5	6	12	11	14	13	7
Left	Right	Right	Left	Right	Right	Right	Right	Left	Right
-	-	-	-	-	-	-	-	-	-
529	582	-	-	-	-	-	-	-	-
24.29	-	-	-	-	-	-	-	-	-

BVCA is in LogMAR units. Rx = refractive error in Dioptres. GAT, RBT_{on} and RBToff are in mmHg. Nystag = nystagmus, Corn def = corneal defect, Oc hist = ocular history, CC = corneal curvature, AL = axial length, CCT = central corneal thickness.

Appendix 3.0: Raw data used for Experiment 4

1.00		8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	HCTD	ID
16.00	Controls	20.00	18.00	10.00	18.00	16.00	16.00	18.00	14.00		RBToff
18.00		20.00	19.00	11.00	22.00	17.00	21.00	19.00	16.00		RBTon
17.00		17.00	15.00	10.00	21.00	14.00	14.00	18.00	14.00		GAT
0.00		17.00	(-9.00/-1.00x180)	-5.50	(-4.00/-7.50x160)	(+1.50/-0.50x175)	-11.00	(+5.00/-9.00x180)	(-3.00/-6.00x180)		Rx
0.00		0.10	0.18	0.46	0.10	0.04	0.55	0.84	0.54		BCVA
none		none	none	none	medical	none	none	none	none		Treatmen
no		yes	no	no	no	no	no	no	no		Corn def
no		no	no	no	no	no	yes	no	yes		Strab
no		no	no	no	no	no	no	no	no		Nystag
Healthy		MFS	MFS	nMFS	MFS	EDS	MFS	MFS	MFS		Med hist
None		EL	Pd	EL (ou)	EL/GL	Healthy	EL	EL	EL		Oc hist
12		10	15	6	10	6	8	6	5		Age
F		F	M	F	F	M	F	M	F		Gender
White		White	Asian	White	White	White	White	White	White		Ethnicity
Left		Left	Left	Right	Left	Right	Right	Left	Left		Eye
-		-	-	-	-	-	-	-	-		CCT (µm)
-		-	-	-	-	-	-	7.69 (180)	-		CC (mm)
-		-	-	-	-	-	23.49	22.98	-		AL (mm)

2.00	18.00	24.00	17.00	3.00	0.10	none	no	no	no	Healthy	None	10	M	White	Left	-	-	-
3.00	10.00	11.00	10.00	0.00	0.08	none	no	no	no	Healthy	None	8	F	White	Right	-	-	-
4.00	13.00	16.00	13.00	(+1.00/-0.75x170)	0.14	none	no	no	no	Healthy	None	6	F	White	Left	-	-	-
5.00	22.00	22.00	18.00	(+0.50/-0.25x93)	0.00	none	no	no	no	Healthy	None	11	M	White	Right	-	-	-
6.00	20.00	17.00	20.00	(+3.25/-2.00x180)	0.00	none	no	no	no	Healthy	None	15	F	Asian	Left	-	-	-
7.00	14.00	15.00	12.00	(+2.00/-0.25x178)	0.00	none	no	no	no	Healthy	None	7	M	White	Right	-	-	-
8.00	18.00	14.00	15.00	(+2.00/-0.25x180)	0.08	none	no	no	no	Healthy	None	6	F	Asian	Right	-	-	-

BVCA is in LogMAR units. Rx = Refractive error in Dioptres. GAT, RBT_{on} and RBToff are in mmHg. Nystag = nystagmus, Strab = strabismus, Med hist = medical history, Treatmen = treatment, Corn def = corneal defect, Oc hist = ocular history, CC = corneal curvature, AL = axial length, CCT = central corneal thickness.

Appendix 4.0: Supporting documents

NHS
Health Research Authority

North West - Liverpool East Research Ethics Committee
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8009

23 March 2016

Dr Leon Davies
School of Life and
Health Sciences
Aston University
Birmingham
B47ET

Dear Dr Davies

Study title:	**The validity and reliability of intraocular pressure measurement using rebound tonometry in young children.**
REC reference:	**16/NW/0237**
Protocol number:	**1**
IRAS project ID:	**186371**

The Proportionate Review Sub-committee of the North West - Liverpool East Research Ethics Committee reviewed the above application on 28 March 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Miss Amber Ecclestone, nrescommittee.northwest-liverpooleast@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion
On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion
The REC favourable opinion is subject to the following conditions being met prior to the start of the study.
Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

There were no ethical issues raised.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Advert]	3	11 November 2015
Covering letter on headed paper [Cover letter]	1	14 March 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors		17 July 2015

only) [Aston EL-PL15]		
GP/consultant information sheets or letters [GP letter]	1	02 June 2015
Instructions for use of medical device [d-Kat instructions]		22 July 2013
Instructions for use of medical device [ICARE manual]		02 April 2012
Letter from sponsor [Aston Gov approval]	1	09 March 2016
Letter from statistician [Statistics]	1	03 August 2015
Letters of invitation to participant [Invitation letter]	1	20 February 2016
Other [Academic Supervisor 2 CV]	1	17 September 2015
Other [Aston medical malpractice mal]		17 July 2015
Other [Aston professional indemnity]		17 July 2015
Other [Participant certificate of achievement]	1	28 December 2015
Other [Key Collaborator CV]	1	04 June 2015
Other [Key Collaborator GCP]		15 June 2013
Other [Key Collaborator GCP consent]		07 March 2016
Other [CI/academic supervisor GCP]		12 November 2014
Other [Academic Supervisor 2 GCP]		22 February 2016
Other [Student GCP]		09 March 2015
Other [Student GCP consent]		06 May 2015
Participant consent form [Consent]	1	02 June 2015
Participant consent form [Assent]	1	02 June 2015
Participant information sheet (PIS) [PIS under 6]	7	19 January 2016
Participant information sheet (PIS) [PIS 6-10]	4	19 February 2016
Participant information sheet (PIS) [PIS 10-14]	4	19 February 2016
Participant information sheet (PIS) [PIS 14-16]	3	19 February 2016
Participant information sheet (PIS) [PIS adult]	2	19 February 2016
REC Application Form [REC_Form_21032016]		21 March 2016
Referee's report or other scientific critique report [Aston project feedback]		19 March 2015
Research protocol or project proposal [Protocol]	4	10 February 2016
Summary CV for Chief Investigator (CI) [Dr Leon Davies CV]	1	10 September 2015
Summary CV for student [Student CV]	1	28 January 2016
Summary CV for supervisor (student research) [Academic Supervisor CV]	1	10 September 2015
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Brief summery]	1	13 March 2016

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance

on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback
The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training
We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

16/NW/0237	**Please quote this number on all correspondence**

Yours sincerely

Signed on behalf
of Alternate Vice-Chair Dr
Peter Walton

Email: nrescommittee.northwest-liverpooleast@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Miss Alpa Patel*

Ms Rachel Rikunenko, Birmingham Children's Hospital NHS Foundation Trust

Research and Development
Steelhouse Lane Birmingham
Our Ref: TM/MS/R&D Approval 20 April 2016

Joseph Abbott
Consultant Paediatric Ophthalmology
Birmingham Children's Hospital,
Steelhouse Lane,
Birmingham, B4 6NH

Dear Joseph

Re: Birmingham Children's Hospital NHS Foundation Trust R&D Approval

Project Title:	**The validity and reliability of intraocular pressure measurement using rebound tonometry in young children**
REC Ref:	**16/NW/0237**
IRAS Project ID:	**186371**

Thank you for informing Birmingham Children's Hospital NHS Foundation Trust's R&D office of the above project.

I am now happy to approve the above research project. You will note from the Research Ethics Committee (REC) approval letter dated 23rd March 2016 that the favourable opinion is subject to obtaining management permission or approval at each host organisation prior to the start of the research project. Approval of the study is subject to the following conditions:

1. That you inform and send copies of correspondence to the R&D Office, the appropriate regulatory authorities and competent authorities of any amendments.
2. That you notify the R&D Office of any adverse events arising from this piece of research in line with Birmingham Children's Hospital R&D Office Pharmacovigilance and Safety Reporting SOP.
3. That you provide the R&D Office with copies of the REC annual progress reports and end of study declaration form as well as any acknowledgements.
4. That you conduct the research in conformity with the Research Governance Framework and other legal and regulatory requirements where applicable.
5. That the chief/Principal Investigator and the research team should be familiar with BCH trial Standard Operating Procedures. These SOPS can be found at the following location on the trust intranet.http://solitaire.zion.matrix.local/corporate/research-and-development/key-documents/r%2526d-useful-documents
6. That any publications arising from this work include appropriate acknowledgements for support provided by, for example, NIHR CRN.

Documents approved:

Documents:	*Version:*	*Date:*
Copies of advertisement materials for research participants [Advert]	3	11 November 2015
GP Letter	1	02 June 2015
Instructions for use of medical device [d-Kat		22 July 2013
Instructions for use of medical device [ICARE manual]		02 April 2012
Invitation Letter	1	20 February 2016
Participant consent form [consent]	1	02 June 2015
Participant consent form [assent]	1	02 June 2015
Participant information sheet (PIS) [PIS under 6]	7	19 January 2016
Participant information sheet (PIS) [PIS 6-10]	4	19 February 2016
Participant information sheet (PIS) [PIS 10-14]	4	19 February 2016
Participant information sheet (PIS) [PIS 14-16]	3	19 February 2016
Participant information sheet (PIS) [PIS Adult]	2	19 February 2016
Research Protocol	4	10 February 2016
Brief Summary	1	13 March 2016

Your research project documents can be found at: V:\R&D Study Head and Neck\Ophthalmology\Abbott_J_1764_RBTStudy. If you cannot access this please let us know and we will provide this.

Please inform the R&D Data Manager via email marie.thomas2@bch.nhs.uk when you have recruited your first patient.

Finally, I would like to take this opportunity to wish you well with your research project. If you need any further assistance or guidance, please do not hesitate to contact us.

Yours sincerely,

[redacted]

Theresa Morton
Head of Research, Development and Innovation

Aston Triangle
Birmingham B4
7ET United
Kingdom

Tel: +44 (0)121 204 3000

www.aston.ac.uk

Nicola Karen Sabokbar

School of Life and Health Sciences

29th April 2016

Dear Karen

Study title:	The validity and reliability of intraocular pressure measurement using rebound tonometry in young children.
REC reference:	16/NW/0237 [North West - Liverpool East Research Ethics Committee]
Protocol number:	1
IRAS project ID:	186371
AHRIC ref number:	117S/NS
NHS Research Site:	Birmingham Children's Hospital, Steelhouse Lane, Birmingham, B4 6NH.

I am writing to confirm permission for your project to proceed on behalf of the University Research Ethics Committee.

This approval is subject to:

- The project being undertaken in conjunction with the NHS site listed above.
- Undertaking the project as described in the Protocol.
- Using the supporting documents listed below.
- Participation of staff and students as described below.
- Formal approval of any amendments' including personnel changes.
- Adverse event and serious adverse event reporting.
- Provision of annual reports.

Amendments to the Project

Any proposed amendments to the project (including personnel) must be approved by AHRIC and if required NHS Research Ethics Committee approval prior to implementation.

Approval of AHRIC should be sought by e-mailing details of the amendment to ahricgovernance@aston.ac.uk.

Adverse Event and Serious Adverse Event Reporting

In addition to any regulatory requirements for reporting adverse events and serious adverse events you are required to submit details of any adverse events to the University Research Ethics Committee.

Details of the adverse event or serious adverse event and any subsequent action should be submitted to John Walter, Secretary to the University Ethics Committee (j.g.walter@aston.ac.uk) within 24 hours of the event occurring.

Reporting Requirements

Continued approval of the project is subject to:

- A copy of the NHS Ethics Committee Annual Report Form being submitted to ahricgovernance@aston.ac.u each year prior to the date of this approval.
- An End of Study Report should be submitted to AHRIC and the NHS Ethics Committee at the point of 'data lock' i.e. a point at which the raw dataset is considered to be accurate (checked for anomalies), cleansed, validated and anonymised and does not require access to Case Report Forms. Note: data analysis can continue after 'data lock'

Research Governance Responsibilities

The research governance responsibilities of those involved in research (as described in the Research Governance Framework and the Good Clinical Practice Guidelines) are outlined in Appendix A.

Failure to comply with the terms of this approval will result in withdrawal of approval and indemnity for the project.

May I take this opportunity to wish you well with your study and please do not hesitate to contact me if you require any further assistance in relation to the Governance or regulatory approvals for this project

Yours sincerely,

Nichola Seare

Chair, Aston University Research Ethics Committee

Approved Documents

NHS Approved Study Documentation	Version Numbe	
Copies of advertisement materials for research participants [Advert]	3	11 November 2015
GP/consultant information sheets or letters [GP letter]	1	02 June 2015
Instructions for use of medical device [d-Kat instructions]		22 July 2013
Instructions for use of medical device [ICARE manual]		02 April 2012
Letter from statistician	1	03 August 2015
Letters of invitation to participant	1	20 February 2016
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Participant information sheet [PIS 10-14]	4	19 February 2016
Participant information sheet [PIS 14-16]	3	19 February 2016
Participant information sheet [PIS adult]	2	19 February 2016
Referee's report or other scientific critique report [Aston project feedback]		19 March 2015
Research protocol	4	10 February 2016
Summary, synopsis or diagram (flowchart) of protocol in Non-technical language [Brief summery]	1	13 March 2016
Summary CV for Chief Investigator (CI) [Dr Leon Davies CV]	1	10 September 2015
Summary CV for student [Nicola Karen Sabokbar]	1	28 January 2016
Summary CV for supervisor [Nicola Logan]	1	10 September 2015
Key Collaborator CV – Joseph Abbott	1	04 June 2015
Key Collaborator GCP - Joseph Abbott		15 June 2013
Key Collaborator GCP consent Joseph Abbott		07 March 2016
CI/academic supervisor GCP – Leon Davies		12 November 2014
Academic Supervisor 2 GCP – Nicola Logan		22 February 2016

Student GCP – Nicola Karen Sabokbar		09 March 2015
Student GCP consent – Nicola Karen Sabokbar		06 May 2015

Other Study Supporting Documentation	
	Favourable Opinion Letter dated 23.03.16 issued by North West – Liverpool East NHS REC
	R&D Permissions letter dated 20.04.16 issued by BCH R&D.
	Letter dated 20.05.16 from Theresa Morton, BCH, confirming Joseph Abbott's substantive contract
	Honorary Contract with Birmingham Children's Hospital (BCH) for: Nicola Karen Sabokbar and Leon Davies
	Aston Governance Form
	Delegation of Duties Log

Project Staff

Name	Approved to take Consent	Approved to handle human tissue
Nicola Karen Sabokbar and Joseph Abbott	Yes	NA
Leon Davies and Nicola Logan	NA	NA

Appendix A

RESPONSIBILITIES UNDER THE RESEARCH GOVERNANCE FRAMEWORK

Chief Investigator Responsibilities

- Developing the protocol, including where possible involving potential participants
- Study management procedures
- Compliance with legal, ethical and research governance requirements
- Ensuring the research team is appropriately qualified to undertake the study
- Protocol amendments, their approval and implementation
- Ensuring participant welfare
- Dissemination, including feeding back results to the participants

Principal Investigator Responsibilities

- The Principal takes overall responsibility for a study at a site. This included but is not restricted to the duties listed below. Some duties may be delegated to other members of the research team but the responsibility remains with the PI. Duties that are underlined and in bold cannot be delegated.
- Negotiation and completion of the financial agreement
- Indemnity, compensation and insurance
- **Delegation of study related duties**
- **Ensuring all staff delegated to work on the study are adequately informed as to protocol requirements and trained in specific procedures**
- Participant recruitment strategy
- Medical care and supervision of patients (if applicable)
- Screening participants for eligibility
- Informed consent process
- Randomization (if applicable)
- **For trials of Investigational Medicinal Products (IMPs):**

Familiarity with the Investigator Brochure

- Administration of Investigational Medicinal Product
- Dispensing (if applicable)
- IMP accountability and monitoring of compliance
- Collection of study-related blood samples (if applicable)
- Completion of data collection forms
- Documenting of Adverse Events (AE)
- Timely reporting of Serious Adverse Events (SAE)
- **Deciding causality and expectedness of SAE**
- Ethics committee approval / communication re: amendments
- Availability for audit and inspections
- Archiving

Researcher Responsibilities

- Ensuring research undertaken follows the current version of the protocol
- Helping care professionals to ensure research patients receive appropriate care whilst involved in research
- Protecting the integrity and confidentiality of clinical and other information generated by the research
- Reporting any adverse events and suspected misconduct

Sponsor Responsibilities

- Assuring scientific quality (peer review)
- Ensuring research ethics committee approval
- Resources and financial management
- Ensuring arrangements for the management and monitoring of research are in place
- Compensation to participants

Employer Responsibilities

- Developing and promoting a highquality research culture – accountability for professional conduct
- Ensuring employees meet obligations set out in law and relevant guidance
- Compliance with employment and health and safety legislation
- Undertaking agreed management and monitoring roles
- Ensuring anyone who is harmed as a result of negligence can be compensated
- Systems to detect and address misconduct and fraud

Care Organisation Responsibilities

- Ensuring that research undertaken in their organisation meets the standards in the Research Governance Framework
- Ensuring ethics committee approval
- Retaining responsibility for research participants' care

ANNUAL PROGRESS REPORT TO MAIN RESEARCH ETHICS COMMITTEE
(For all studies except clinical trials of investigational medicinal products)

To be completed in typescript and submitted to the main REC by the Chief Investigator. For questions with Yes/No options please indicate answer in bold type.

1. Details of Chief Investigator

Name:	Dr Leon Davies
Address:	School of Life and Health Sciences Aston University Birmingham B4 7ET
Telephone:	0121 204 4152
E-mail:	l.n.davies@aston.ac.uk
Fax:	0121 204 4048

2. Details of study

Full title of study:	The validity and reliability of intraocular pressure measurement using rebound tonometry in young children,
Name of main REC:	North West – Liverpool East Research Ethics Committee
REC reference number:	16/NW/0237
Date of favourable ethical opinion:	23rd March 2016
Sponsor:	Dr Nichola Seare

3. Commencement and termination dates

Has the study started?	***Yes***
If yes, what was the actual start date?	31st May 2016
If no, what are the reasons for the study not commencing? What is the expected start date?	
Has the study finished?	***No***

If yes, complete and submit "Declaration of end of study" form, available at http://www.nres.npsa.nhs.uk/applications/after-ethical-review/endofstudy/	

If no, what is the expected completion date? If you expect the study to overrun the planned completion date this should be notified to the main REC for information.	**30th Sept 2018**
If you do not expect the study to be completed, **give reason(s)**	

4. Registration

Is the study a 'clinical trial'? (Defined as first 4 categories on the IRAS filter page) (For CTIMP please use CTIMP progress reporting template)	***No***
Is the study registered on a publically accessible database? (Registration of clinical trials is a condition of approval for studies approved after 30 September 2013)	**No**
If yes, please provide the name of the database and the registration number Database: Registration number:	
If no: a. What is the reason for non-registration? This study is not a clinical trial b. What are your intentions for registration? I am not intending to register	

5. Site information

Do you plan to increase the total number of sites proposed for the study? If yes, how many sites do you plan to recruit?	No

6. Recruitment of participants

In this section, "participants" includes those who will not be approached but whose samples/data will be studied.

Number of participants recruited: 35	Proposed in original application: 50 *Actual number recruited to date: 35*
Number of participants completing trial: 35	*Actual number completed to date: 35*
Number of withdrawals from study to date due to: (a) withdrawal of consent 0 (b) loss to follow-up 0 (c) death (where not the primary outcome) 0 Total study withdrawals: 0	
*Number of treatment failures to date (prior to reaching primary outcome) due to: (a) adverse events (b) lack of efficacy Total treatment failures: * Applies to studies involving clinical treatment only	
Have there been any serious difficulties in recruiting participants?	**No**
If Yes, give details:	
Do you plan to increase the planned recruitment of participants into the study? *Any increase in planned recruitment should be notified to the main REC as a substantial amendment for ethical review.*	**No**

7. Safety of participants

Have there been any related and unexpected serious adverse events (SAEs) in this study?	No
Have these SAEs been notified to the Committee? *If no, please submit details with this report and give reasons for late notification.*	**Not applicable**

Have any concerns arisen about the safety of participants in this study? *If yes, give details and say how the concerns have been addressed.*	***No***

8. Amendments

Have any substantial amendments been made to the trial during the year?	No
If yes, please give the date and amendment number for each substantial amendment made.	

9. Serious breaches of the protocol

Have any serious breaches of the protocol occurred during the year? *If Yes, please enclose a report of any serious breaches not already notified to the REC.*	***No*** ***N/A***

10. Other issues

Are there any other developments in the study that you wish to report to the Committee? Are there any ethical issues on which further advice is required? *If yes to either, please attach separate statement with details.*	***No*** ***No***

11. Declaration

Signature of Chief Investigator:	[redacted]
Print name:	Leon Davies
Date of submission:	7th April 2017

Reply from North West REC to first year report:

North West - Liverpool East Research Ethics Committee Barlow House 3rd Floor 4 Minshull Street Manchester M1 3DZ

08 May 2017

Dr Leon Davies School of Life and Health Sciences Aston University Birmingham B4 7ET

Dear Dr Davies

Study Title: The validity and reliability of intraocular pressure measurement using rebound tonometry in young children. REC reference: 16/NW/0237 Protocol number: 1 IRAS project ID: 186371

Thank you for sending the progress report for the above study dated 07 April 2017. The report will be reviewed by the Chair of the Research Ethics Committee, and I will let you know if any further information is requested.

The favourable ethical opinion for the study continues to apply for the duration of the research as agreed by the REC.

16/NW/0237: Please quote this number on all correspondence

Yours sincerely

Damilola Odunlami

E-mail: nrescommittee.northwest-liverpooleast@nhs.net

End of study report for Northwest Liverpool REC:

The validity and reliability of intraocular pressure measurement, using rebound tonometry in young children

Nicola Sabokbar[1], Joseph Abbott[2], Nicola Logan[1], Leon Davies[1]
[1]Aston Optometry School, Aston University
[2]Birmingham Women's and Children's Hospital (BWCH)

Summary of final research report: 16/NW/0237

Objective: Three main objectives: 1. The validity of Rebound Tonometry (RBT) measurements in children. 2. The reliability of suboptimal RBT readings and the relationship between co-existing characteristics and these measurements. 3. The reliability of suboptimal RBT measurements in children with heritable connective tissue disease (HCTD).
Design: A cross-sectional study design was used for objectives 1 and 2 and a case control study was used for objective 3.
Setting: The Eye Department of Birmingham Women's and Children's Hospital (BWCH).
Participants: A total of 50 children were recruited including 34 with glaucoma for objectives 1 and 2 and 16 for objective 3 (8 with HCTD, 8 healthy controls).
Interventions: RBT measurements were taken at the geometric centre of the cornea of one eye (RBT_{on}) and at 3 mm temporally (RBT_{off}), followed by Goldmann tonometry (GAT). Additional data regarding sex, age, nystagmus, strabismus, type of glaucoma, treatment, visual acuity, spectacle prescription, ethnicity, health and corneal scars were recorded from the participants' clinical notes. The same procedure was conducted on 8 children with HCTD and 8 controls
Outcome measures: Objective 1 was analysed by comparing RBT_{on} and GAT measurements taken at the centre of the cornea. In addition, the association of co-existing characteristics and the difference between these measurements was examined. The second objective was analysed by comparing suboptimal RBT_{off} with RBT_{on} and looking at the association of the co-existing characteristics. Objective 3 was analysed by comparing RBT_{off} and RBT_{on} in children with HCTDs, with similar measurements in age matched healthy controls.
Results: Recruitment rate was 89% due to some participants being ineligible or declining to participate. Mean RBT_{on} was significantly higher than GAT and a statistical difference was found between the age groups and the IOP status ($p < 0.05$). Mean RBT_{off} readings were not significantly different from RBT_{on} in children with glaucoma ($p = 0.100$) and this difference was not associated with co-existing characteristics ($p > 0.05$). Mean (RBT_{off} - RBT_{on}) was not significantly different between children with HCTDs and healthy controls and ($p = 0.06$).
There were no adverse events associated with this trial.
Conclusion: This study achieved its main objectives and found that:

- RBT_{on} measurements differ from GAT but are useful clinically.
- The relationship between RBT_{on} and GAT varies with the age of the child.
- Suboptimal RBT_{off} measurements are reliable in children with glaucoma with a range of co-existing conditions and in children with HCTDs.

Dissemination: The results of this study have been presented in poster form at the 2018 UKEGS conference and as an oral presentation at the 2019 UKPGS conference. Two research papers are being prepared for publication. A short summary of the results has been prepared for display in the eye department of BWCH.

End of study confirmation from Northwest Liverpool REC:

From: nrescommittee.northwest-liverpooleast@nhs.net [mailto:noreply@harp.org.uk]
Sent: 27 April 2018 11:26
To: Davies, Leon N <l.n.davies@aston.ac.uk>; sabokbarfamily@hotmail.com
Cc: Patel, Alpa <A.PATEL10@aston.ac.uk>; rachel.rikunenko@nhs.net
Subject: IRAS PROJECT ID 186371, REC Reference 16/NW/0237 : Acknowledgement of end of study

Dear Dr Davies

Study title:	**The validity and reliability of intraocular pressure measurement using rebound tonometry in young children.**
REC reference:	**16/NW/0237**
Protocol number:	**1**
IRAS project ID:	**186371**

Thank you for sending the declaration of end of study form, notifying the Research Ethics Committee that the above study concluded on 27 April 2018. I will arrange for the Committee to be notified.

A summary of the final research report should be provided to the Committee within 12 months of the conclusion of the study. This should report on whether the study achieved its objectives, summarise the main findings, and confirm arrangements for publication or dissemination of the research including any feedback to participants.

16/NW/0237 Please quote this number on all correspondence

Yours sincerely

Sean Price

Health Research Authority

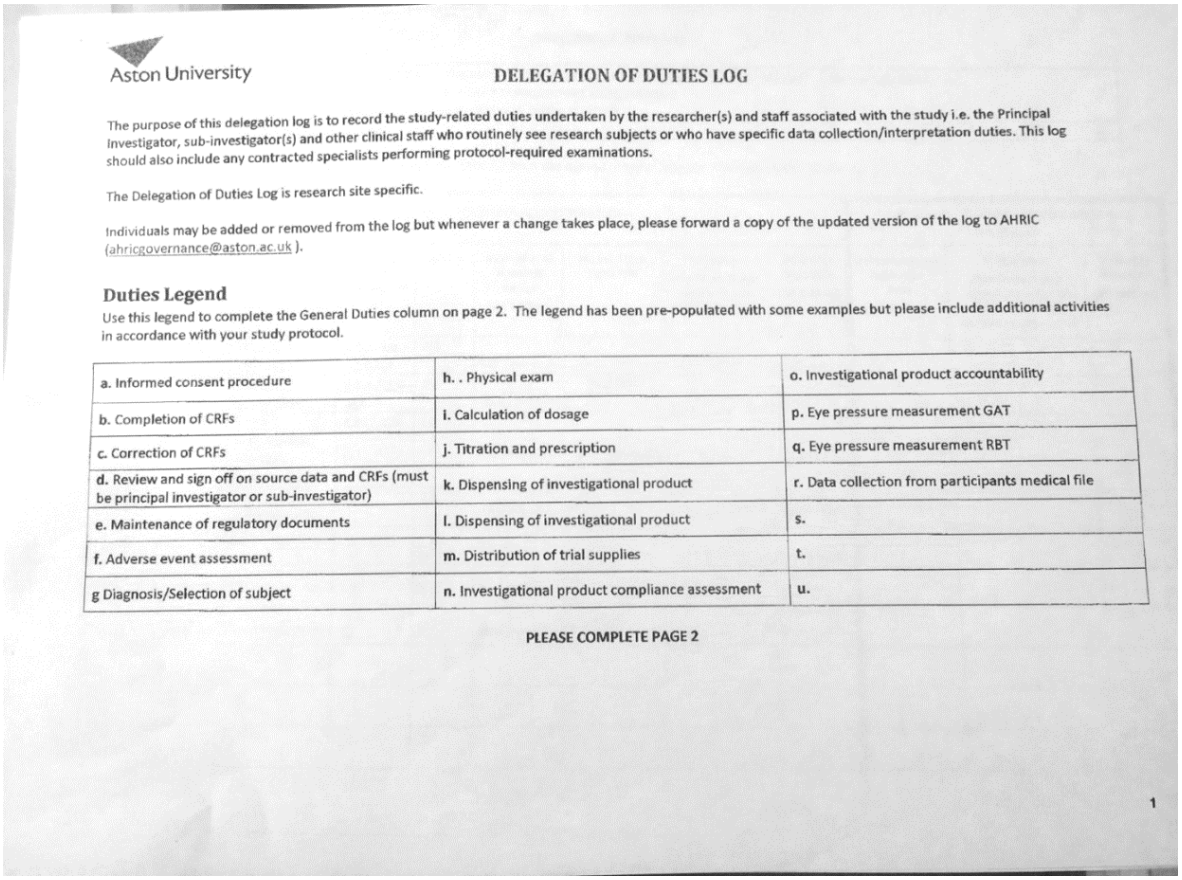
Barlow House | 3rd Floor | HRA NRES Centre Manchester | M1 3DZ

T.

E. nrescommittee.northwest-liverpooleast@nhs.net

W. www.hra.nhs.uk

Delegation of Duties Log:



Aston University

DELEGATION OF DUTIES LOG

The purpose of this delegation log is to record the study-related duties undertaken by the researcher(s) and staff associated with the study i.e. the Principal Investigator, sub-investigator(s) and other clinical staff who routinely see research subjects or who have specific data collection/interpretation duties. This log should also include any contracted specialists performing protocol-required examinations.

The Delegation of Duties Log is research site specific.

Individuals may be added or removed from the log but whenever a change takes place, please forward a copy of the updated version of the log to AHRIC (ahricgovernance@aston.ac.uk).

Duties Legend

Use this legend to complete the General Duties column on page 2. The legend has been pre-populated with some examples but please include additional activities in accordance with your study protocol.

a. Informed consent procedure	h. . Physical exam	o. Investigational product accountability
b. Completion of CRFs	i. Calculation of dosage	p. Eye pressure measurement GAT
c. Correction of CRFs	j. Titration and prescription	q. Eye pressure measurement RBT
d. Review and sign off on source data and CRFs (must be principal investigator or sub-investigator)	k. Dispensing of investigational product	r. Data collection from participants medical file
e. Maintenance of regulatory documents	l. Dispensing of investigational product	s.
f. Adverse event assessment	m. Distribution of trial supplies	t.
g Diagnosis/Selection of subject	n. Investigational product compliance assessment	u.

PLEASE COMPLETE PAGE 2

1

Aston University

Birmingham Children's Hospital NHS
NHS Foundation Trust

Aston University Research study looking into a new way of measuring eye pressure without using eye drops.

We want to understand more about

A new way of measuring eye pressure. Eye pressure measurements are taken to check for glaucoma.

The most common eye pressure test is Goldman Applanation Tonometry (GAT), which involves the use of eye drops to numb the eyes before the test takes place.

But there is a new test called the rebound tonometer (RBT) which does not require the use of eye drops.

We would like to compare RBT with GAT. One GAT measurement and two RBT measurements will be taken from one eye. The measurements we obtain will help us to see how accurate RBT is. This can be undertaken at a routine eye appointment and will only take a few minutes.

We are hoping you can help	If your child has eye pressures measured at routine appointments at Birmingham Children's Hospital Eye Department
Please contact the principle researcher Nicola Sabokbar for futher information. Email: sabokban@aston.ac.uk Tel: 07555351003	and is willing to have 1 GAT test and 2 RBT tests-it will only take a few minutes

Advert Version 3, date 30/11/2015

Invitation

The validity and reliability of rebound tonometry in young children. (RBT Study).

Hi, my name is Nicola Sabokbar and I am carrying out a research project as part of my doctorate at Aston University. I would like to invite your child to take part.

The reason why I am carrying out this project is because sometimes, we need to measure children's eye pressures when they come to the hospital for a check- up.

This research project looks at a new way of measuring eye pressure without using eye drops. I want to compare the standard way of measuring eye pressure, Goldman Applanation tonometry (GAT) with the new way (Icare rebound tonometer (RBT), to see which is best.

Your child has been chosen because he/she is between1 month and 16 years old and needs to have his/her eye pressures measured as part of their care at Birmingham Children's Hospital.
It is up to you and your child whether he/she wants to take part and your child can always change his/her mind
We cannot promise that the study will help your child but the information we get might help other young people with glaucoma and connective tissue disorders in the future.

RBT measurements will be taken first followed by GAT. The whole procedure will take about ten minutes. One of these measurements might be taken as part of your child's routine care.
With permission from you and your child other data obtained from your child's medical notes will be used to see if it has any relationship with RBT readings.
Your child will be given a certificate for taking part.

Your healthcare team will ask you and your child if you are interested in taking part.
If you are interested you will be given more information.

Thank you
Nicola Sabokbar
Invitation (1), date 20th Feb 2016

Letter to patient`s GP

Date

Dear Dr

Re:

…….
…….

The validity and reliability of rebound tonometry in young children (RBT Study)

Reference No.

I am writing to inform you that your patient has been enrolled into the above research study.

The purpose of the study is to examine the validity and reliability of rebound tonometry in young children, as very little is known about this. This involves measuring eye pressures with Goldman applanation tonometry (GAT) and with rebound tonometry (RBT) during a routine appointment at Birmingham Children's Hospital. The results will be published in scientific journals.

A copy of the patient information sheet is attached.

If you have any questions regarding any of the above, please feel free to email me at **sabokban@aston.ac.uk** .

Yours sincerely

Nicola Sabokbar

Optometrist

GP letter Version 1, date 2 Jun 2015

Certificate of achievement

Has earned this certificate for taking
Part in the RBT eye study!

Well done!

Mrs Nicola Sabokbar
(Principal Investigator)

The validity and reliability of rebound tonometry in young children. (RBT Study)

Child/ young person assent form

Study ID……

Patient ID…… **Patient Initials……. Date of birth**……

Child to circle all they agree with

Have you read (or had read to you) information about this project?	Yes/No
Has somebody else explained this project to you?	Yes/No
Do you understand what this project is about?	Yes/No
Have you asked the questions you want?	Yes/No
Have you had your questions answered in a way you understand?	Yes/No
Do you understand its ok to stop taking part at any time?	Yes/No
Are you happy to begin this study?	Yes/No

If any answers are "no" and you don't want to take part, do not sign your name.
If you do want to take part in this study, please sign your name and write today's date.

Your name……..
Date…….

The researcher who explained this project to you needs to sign here too:

Researcher……**Signature**……..**Date**……
Thank you for your help

ASS Version 1, date 2 Jun 2015

The validity and reliability of rebound tonometry in young children. (RBT Study)

Parent/Guardian consent form

Study ID……

Patient ID…… **Patient Initials…….. Date of birth**……

Please initial the boxes:

1. I confirm that I have read and understood the information sheet attached……. (version……) for the above study. I have had the opportunity to consider the information, ask questions and they have answered these satisfactorily.	
2. I understand that my child's participation is voluntary and that he/she is free to withdraw at any time, without giving a reason and without my child's care or legal rights being affected.	
3. I understand that relevant sections of any of my child's medical notes and data collected during the study may be looked at by responsible individuals from the Sponsor organization, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in the research. I give permission for these individuals to have access to my child's records.	
4. I agree to my child's GP being informed of my child's participation in the study.	
5. I consent to the storage including electronic of personal information for the purposes of this study. I understand that any information that could identify my child will be kept strictly confidential and that no personal information will be included in the study report or other publication.	

Name of Child: ……

Name of Parent/Guardian …… **Signature ……. Date**

Researcher …… Signature …… Date

Enquiries:
Tel: **07555 351003**
Email: **sabokban@aston.ac.uk**

Principal Investigator
Nicola Sabokbar
Birmingham Children's Hospital NHS Trust

Steelhouse Lane, Birmingham, B4 6NH

CON Version 1, date 2 Jun 2015

Aston University

Birmingham Children's Hospital NHS
NHS Foundation Trust

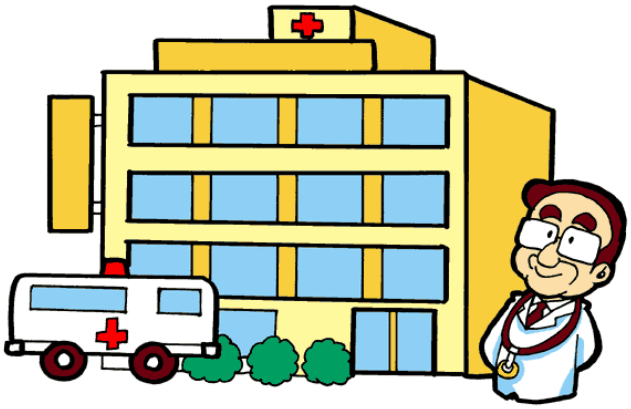
Participant information sheet (under 6 years)

The validity and reliability of rebound tonometry in young children.
(RBT Study).

This leaflet is intended to be shown/read to the child by their parent/guardian

Hello, my name is Nicola.

I work in a hospital.



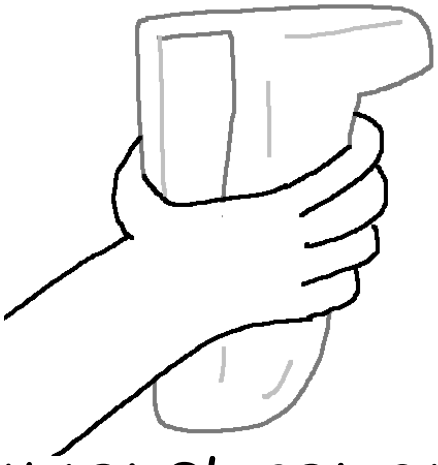
At the hospital, I check children's eyes to make sure they are working properly.

I would like to invite you to have your eyes checked.

And, if you are happy to let me check your eyes, you will visit me at the hospital

I will give you some glasses to wear.

Then I will use something that looks this



And gently touch something like a feather onto your eyes. It will feel like someone is tickling your eyes – it won't hurt.

Then I will put some drops in your eyes and check your eyes with something else that looks similar. – your eyes may feel watery but it won't hurt.

After I have checked your eyes you will get a certificate, and it will look something like this.

If you don't want to have your eyes checked, it is ok to say NO.

Contact for further information

Chief Investigator: Dr Leon Davies
Telephone: [redacted], email: **l.n.davies@aston.ac.uk**

Researcher: Mrs Nicola Sabokbar
Telephone: [redacted], email: **sabokban@aston.ac.uk**

Clinical Supervisor: Mr Joseph Abbott
Telephone: [redacted], email: **Joseph.Abbott@bch.nhs.uk**

Thank you for your time and thinking about taking part in the study.

PIS <6 (7), date 19th Jan 2016

Participant information sheet (age 6-10 years)

The validity and reliability of rebound tonometry in young children (RBT study)

Invitation

Hi, my name is Nicola Sabokbar and I am carrying out a research project as part of my degree. I would like to invite you to take part in my research project.

Before you decide if you want to join in, it is important to understand what the project is about and what it will mean if you take part. So please read this leaflet carefully with your parents. Also talk to your family, friends, doctor, nurse or a member of the research team whose details are at the end of this information sheet.

What is research?

Research is a way of finding the answers to questions.

Why is this project being done?

This study is about a new way of measuring eye pressure without using eye drops.

The reason why I am carrying out this project is because sometimes, we need to measure children's eye pressures when they come to the hospital for a check- up.

We measure eye pressure because some children have a disease called glaucoma.

We use tools called tonometers to measure eye pressure and for this project I want to compare two tonometers which are called:

1. Goldman Applanation Tonometer (GAT)
2. Rebound tonometer (RBT)

We want to compare both the GAT and RBT to find out which is the best way of measuring eye pressure.

Why have I been asked to take part?
You have been asked because you are 6 to10 years old and need to have your eye pressures measured as part of your care at Birmingham Children's Hospital.

Did anyone else check the study is ok to do?
Before a research project can start, it has to be checked by a group of people to make sure that the research is fair.

Do I have to take part?
No, you don't. It is your choice whether you want to take part and you can always change your mind. If you do not want to take part just tell your parents, doctor or me (Nicola). You don't have to give any reason. **It is YOUR choice.**

What will happen to me if I take part?
You will have your eye pressures measured three times in one eye.
First you will be given some glasses to wear. Then you will have your eye pressures measured with an RBT in two ways. One in the middle of your eye and the other to the side. A small probe will gently touch your eye. Most people do not feel anything, some people say it tickles, but you will not feel any pain.

Then some drops will be put in your eyes. The drops are used to numb your eyes temporarily. They are not painful, but your eyes may water a little.
Then your eye pressures will be measured using the second instrument. You may have had your eye pressures measured before, so you may remember what that was like. One of these measurements might be part of your routine care.

With your permission we will check your medical notes and record some extra measurements, like what you can see and how long your eyes are, that will help us with our research.
We will give you a certificate for taking part.

What if I want to stop taking part?
If you want to stop taking part, just let me know and I will stop the tests and no one will be upset with you.

Could anything about the research upset me?
Eye pressure measurement tests are very safe. Some eye drops will be put in your eyes but none of the measurements will be uncomfortable.

Will my medical details be kept private if I take part? Will anyone else know I'm doing this?
All your information will be kept private. This means we will only tell those who have a need or right to know, like your parents and your GP.

Will joining in help me?
We cannot promise the study will help you but the information we get might help young people with glaucoma and connective tissue disorders in the future. You can ask your doctor, nurse or Nicola Sabokbar for information about the results, when the study has finished.

What happens when the research project finishes?
The research will be talked about and written down but no one will know that you took part.

Where can I find independent information about taking part in research?
You can contact the NHS Patient Advisory Liaison Service (PALS) at Birmingham Children's Hospital if you would like advice on taking part in research: Email: pals@bch.nhs.uk or telephone: 0121 333 8403

Who can I contact if I have any concerns?
If you have any concerns about the way in which the study is being carried out you should first contact me Nicola Sabokbar or my supervisors. All our contact details can be found at the end of this information sheet. If we are unable to help you, you can contact the Secretary to Aston University Ethics Committee, Mr John Walter:
Email: j.g.walter@aston.ac.uk or telephone 0121 204 4869.

Contact for further information

Mrs Nicola Sabokbar
Telephone: [redacted], email: **sabokban@aston.ac.uk**

Chief Investigator: Dr Leon Davies
Telephone: [redacted], email: **l.n.davies@aston.ac.uk**

Clinical Supervisor: Mr Joseph Abbott
Telephone: [redacted], email: **Joseph.Abbott@bch.nhs.uk**

Thank you for reading the information sheet and considering taking part in this study.

PIS 6-10 (4), date 19th Feb 2016

Aston University

Birmingham Children's Hospital NHS
NHS Foundation Trust

Participant information sheet (age 10-14)

The validity and reliability of rebound tonometry in young children. (RBT Study).

Invitation
Hi, my name is Nicola Sabokbar and I am carrying out a research project as part of my degree. I would like to invite you to take part in my research project.

Before you decide if you want to join in, it is important to understand what the project is about and what it will mean if you take part. So please read this leaflet carefully with your parents. Also talk to your family, friends, doctor, nurse or a member of the research team whose details are at the end of this information sheet.

What is research?
Research is a way of finding the answers to questions.

Why are we doing this research?
This research project is about a new way of measuring eye pressure without using eye drops.

The reason why I am carrying out this project is because sometimes, we need to measure children's eye pressures when they come to the hospital for a check- up.

It is important that we measure eye pressure to check if the eyes are healthy.

Eye pressure can be measured in different ways. For this project I want to look at two ways of measuring eye pressure, Goldman Applanation tonometry (GAT) and the Icare rebound tonmeter (RBT).

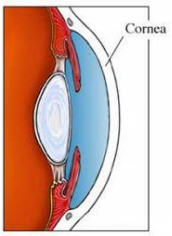
By comparing the GAT measurements with RBT measurements I will be able to find out which one is the best.

Why have I been invited to take part?
You have been chosen because you are 10 to14 years old and need to have your eye pressures measured as part of your care at Birmingham Children's Hospital.

Do I have to take part?
No. It is up to you. It is your choice whether you want to take part and you can always change your mind

What will happen to me if I take part?
During your routine visit to the Eye Department you will be invited to join the study. You can ask any questions you like and if you are happy to join the study, at least one of your parents will need to sign a Consent form to say that you are happy to take part in the study.

Because we want to compare to ways of measuring eye pressure, we will first ask you to have three eye pressure measurements taken using the RBT. RBT takes eye pressure readings from the middle of the cornea.



The cornea is the clear part of the front of your eye.

So, you will be given some glasses to wear then the RBT tonometer will measure your eye pressure at the centre of your eye. This has a small electromagnetic probe that gently touches your cornea six times, measuring your eye pressure. No drops are needed to do this as it is not uncomfortable. Some people feel nothing and others say it tickles a bit.

Then the same rebound tonometer will be moved to a different position, so that the probe can touch a different area of your eye. Another six readings will be taken. This will feel the same as the first readings. The readings only take a few seconds.

We then want to check your eye pressure using the Goldman applanation tonometer.
First some eye drops will be put in your eyes to make them numb. They do not sting, but may make your eyes water a little. This is done so that you will

not feel anything when the measurement is taken. A small cone is used to touch your eye once to take the reading. This procedure will take about five minutes.

RBT measurements will be taken first followed by GAT. The whole procedure will take about ten minutes. One of these measurements might be taken as part of your routine care.

With your permission other data obtained from your medical notes will be used to see if it has any relationship with RBT readings.
You will be given a certificate for taking part.

Is there anything I should be worried about if I take part?
Eye pressure measurements are safe and carried out all the time in hospitals and at the opticians. As part of the study we will use some eye drops to numb your eyes and some other drops to stain your tears yellow. Very occasionally some people can be allergic to these drops, so their eyes can be itchy for a short while afterwards, but this is not serious. If this happens a doctor can prescribe a medicine to sort it out.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we get might help other young people with glaucoma and connective tissue disorders in the future.

Will anyone else know I'm doing this?
We will keep your information private. We will only inform those who have a need or right to know like your parents and your GP.

What will happen to the results of the study?
The results of the research will be published in scientific journals. It will not be possible to identify anyone from the data. You can ask your doctor, nurse or Nicola Sabokbar for information about the results, when the study has finished.

Who is organising and funding the research?
This project is being organised and funded by Aston University. Nicola Sabokbar is the Principal Investigator. She is an optometrist and is undertaking the project as part of her Ophthalmic Doctorate qualification.

Who has reviewed the study?
Before any research goes ahead it has to be checked by an NHS Research Ethics Committee. They make sure that the research is fair. Your project has obtained Aston University Governance Approval and has been checked by the R&D department of Birmingham Children's Hospital.

Where can I find independent information about taking part in research?
You can contact the NHS Patient Advisory Liaison Service (PALS) at Birmingham Children's Hospital if you would like advice on taking part in research: Email: pals@bch.nhs.uk or telephone: 0121 333 8403

Who can I contact if I have any concerns?
If you have any concerns about the way in which the study is being carried out you should first contact me Nicola Sabokbar or my supervisors. All our contact details can be found at the end of this information sheet. If we are unable to help you, you can contact the Secretary to Aston University Ethics Committee, Mr John Walter:
Email: j.g.walter@aston.ac.uk or telephone 0121 204 4869.

Contact for further information

Mrs Nicola Sabokbar
Telephone: [redacted], email: **sabokban@aston.ac.uk**
Chief Investigator: Dr Leon Davies
Telephone: [redacted], email: **l.n.davies@aston.ac.uk**
Clinical Supervisor: Mr Joseph Abbott
Telephone: [redacted], email: **Joseph.Abbott@bch.nhs.uk**

Thank you for reading the information sheet and considering taking part in this study.

PIS 10-14 (4), date 19th Feb 2016

Aston University

Birmingham Children's Hospital NHS
NHS Foundation Trust

Participant information sheet (age 14-16)

The validity and reliability of rebound tonometry in young children.
(RBT Study).

Invitation

Hi my name is Nicola Sabokbar and I am carrying out a research project as part of my degree. I would like to invite you to take part in my research project.

Before you decide if you want to join in, it's important to understand why the research is being done and what it will involve for you. So please consider this leaflet carefully. Also talk to your family friends, doctor, nurse or a member of the research team whose details are at the end of the information sheet.

What is research?

Research is a way of finding answers to questions.

Why are we doing this research?

This research project is about a new way of measuring eye pressure without using eye drops.

The reason why I am carrying out this project is because sometimes, we need to measure children's eye pressures when they come to the hospital for a check- up.

It is important that we measure eye pressure to check if the eyes are healthy.

Eye pressure can be measured in different ways. For this project I want to look at two ways of measuring eye pressure, Goldman Applanation tonometry (GAT) and the Icare rebound tonmeter (RBT).

By comparing the GAT measurements with RBT measurements I will be able to find out which one is the best.

Why have I been invited to take part?

You have been chosen because you are 14 or 15 years old and need to have your eye pressures measured as part of your care at Birmingham Children's Hospital.

Do I have to take part?
No. It is up to you. It is your choice whether you want to take part and you can always say no.

What will happen to me if I take part?
We will ask you to have three eye pressure measurements on one eye.

1. An RBT tonometer will measure your eye pressure at the centre of your eye. This has a small electromagnetic probe that gently touches your cornea six times, measuring your eye pressure. You will be asked to wear a special pair of glasses during the measurement. No drops are needed to do this as it is not uncomfortable.

2. You will be asked to keep the glasses on. The same RBT tonometer will be moved to a different position, so that the probe can touch a different area of your eye. Another six consecutive readings will be taken. Again this will not be uncomfortable.

3. GAT tonometer. First some eye drops with a local anaesthetic will be put in your eyes so you don't feel anything. Then some fluorescein drops will also be put in your eye to make your tears yellow. After that a blue cone is used to touch each eye to measure the pressure.

The whole procedure should take about ten minutes. One of the measurements may be taken as part of your standard care.
With your permission other data like, length of the eye, corneal thickness and general/eye health, obtained from your medical notes will be used to see if it has any relationship with RBT readings.
You will be given a certificate for taking part.

Is there anything I should be worried about if I take part?
Eye pressure measurement is carried out all the time in hospitals and at the opticians and is very safe. As part of the study we will use some eye drops which may cause minor discomfort, but no pain. Details of what you should expect are listed below.
Proxymetacaine 0.5% is a mild local anaesthetic used to numb the surface of the eye. The drops take about 60 seconds to work and around twenty five minutes to wear off. You should not wear contact lenses for thirty minutes and should avoid situations where you might get dust in your eyes as you will not be able to feel it. If you experience unusual symptoms like pain, soreness or blurred vision please contact Nicola Sabokbar (details below) or your GP/optometrist as you may be having an adverse reaction to the drops. (Very occasionally people can be allergic to the eye drops, but this would be unusual).
Fluorescein 1% is used to stain the front of the eye to help read the pressure. It can sting a little when first applied and will make your eye look

slightly orange, but this will not last long and can be washed away with cold water. If you wear contact lenses you will be advised not to wear them for at least fifteen minutes to stop the lenses absorbing the stain.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we get might help us to see how reliable your eye pressure measurements are. Knowing this will help other young people who need to have their eye pressures measured by RBT in the future.

Where can I find independent information about taking part in research?
You can contact the NHS Patient Advisory Liaison Service (PALS) at Birmingham Children's Hospital if you would like advice on taking part in research: Email: pals@bch.nhs.uk or telephone: 0121 333 8403

What happens if there is a problem or something goes wrong?
If you want to make a complaint or give feedback about the study, you can contact the researchers named at the end of this information sheet. They will do their best to address your concerns.
If they are unable to resolve the concerns you raise you can contact the secretary to the Aston University Ethics Committee – Mr John Walter – on **j.g.walter@aston.ac.uk** or telephone 0121 204 4869.

Will anyone else know I'm doing this?
We will keep your information in confidence. This means we will only tell those who have a need or right to know. We will only send out information that has your name and address removed. With your permission we will notify your GP that you are taking part in the study. Your data will be kept securely for fifteen years and will then be destroyed.

What will happen to the results of the study?
The results of the research will be published in scientific journals. It will not be possible to identify anyone from the data. You can ask your doctor, nurse or Nicola Sabokbar for information about the results, when the study has finished.

Who is organising and funding the research?
This project is being organised and funded by Aston University. Nicola Sabokbar is the Principal Investigator. She is an optometrist and is undertaking the project as part of her Ophthalmic Doctorate qualification.

Who has reviewed the study?
Before any research goes ahead it has to be checked by an NHS Research Ethics Committee. They make sure that the research is fair. Your project has obtained Aston University Governance Approval and has been checked by the R&D department of Birmingham Children's Hospital.

Contact for further information

Mrs Nicola Sabokbar
Telephone: [redacted], email: **sabokban@aston.ac.uk**

Chief Investigator: Dr Leon Davies
Telephone: [redacted] email: **l.n.davies@aston.ac.uk**

Clinical Supervisor: Mr Joseph Abbott
Telephone: [redacted], email: **Joseph.Abbott@bch.nhs.uk**

Thank you for reading the information sheet and considering taking part in this study.

PIS 14-16 (3), date 19th Feb 2016

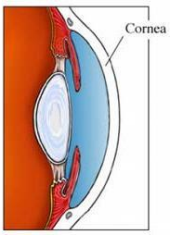
Participant information sheet (Parent/guardian)

The validity and reliability of rebound tonometry in young children. (RBT study).

Invitation
We would like to invite your child to join our research study. This is being undertaken by Nicola Sabokbar in part fulfilment of her Doctorate award, which she is undertaking at Aston University.

Before you decide whether your child can join, it is important to understand why the research is being done and what it will involve for you. If you have any questions or require any further information please ask a member of the research team whose details are at the end of this information sheet.

Why are we doing this project?
This study looks at how accurate eye pressure measurement with a new instrument called the rebound tonometer (RBT) is in young children.
It is important to measure eye pressure in children who may have an eye disease called glaucoma. Glaucoma can be caused by high eye pressure which can lead to irreversible damage to the optic nerve in the eye. Reducing the pressure on the optic nerve is the best way to treat glaucoma.
Usually eye pressure is measured by the Goldman applanation tonometer (GAT). This method uses eye drops to numb the eye which some children dislike. The RBT is a relatively new device that measures the eye pressure without any drops. This device has a small probe that lightly touches the centre of the cornea without causing any discomfort so no numbing drops are needed. As it is a new device we need to compare it with the GAT to see if it is accurate. Children tend to look up when eye pressures are measured, so the RBT measurement may not always be measured at the centre of the cornea.



We would like to see whether these “off-centre” measurements affect the accuracy of RBT readings in children.
Connective tissue disorders like Stickler’s, Alport, Ehler’s Danlos and Marfan’s syndromes etc can affect the eye. We do not know how or if this affects RBT readings, so we would like to investigate this.
With your permission, additional data concerning your child’s eyes will also be collected from medical notes (where possible) in order to examine the influence of other factors like vison, length of the eye, corneal thickness and general/eye health, on eye pressure.

Why has your child been invited to take part?
Your child has been chosen because he/she is between 1 month and 16 years old and needs to have his/her eye pressures measured as part of his/her care at Birmingham Children's Hospital. He/she may either have glaucoma, a connective tissue disorder, or have no eye disease. (No eye surgery/no eye drops/no high prescription).

Does your child have to take part?
No. It is up to you and your child. To enable your child to understand what the study is about and what it involves, we have developed a simplified information sheet for your child to read. If he/she is not at a reading age, we would be grateful if you could read the information sheet to your child. Your child is free to stop taking part at any time during the research without giving a reason. If he/she decides to stop, this will not affect the care received.

What will happen to your child if he/she takes part?
We will ask your child to have three eye pressure measurements taken on one eye.

1. A rebound tonometer will measure your child's eye pressure at the centre of the eye. This has a small electromagnetic probe that gently touches the cornea six times, measuring the eye pressure. Your child will be asked to wear a special pair of glasses during the measurement which will help to position the tonometer correctly. No anaesthetic eye drops are needed to do this as it is not uncomfortable.

2. The glasses will be adjusted and the same rebound tonometer will be moved to a different position so that the probe can touch a different area of the eye. Another six consecutive readings will be taken. Again this will not be uncomfortable.

3. Goldman applanation tonometer. First some eye drops with a local anaesthetic will be put in the eye to numb the front of the eye. Then some fluorescein drops will also be put in the eye to temporarily stain the eye yellow. After that a disposable Goldman tonometer cone will touch the front of the eye to measure the pressure. This will not cause discomfort.

The whole procedure should take about ten minutes. One of these measurements may be taken as part of your child's routine care.
Other data (age, sex, ethnicity, vision, spectacle prescription, binocular vision problems, length and curvature of the eye, corneal thickness/problems, eye disease/ type of treatment and general health) will be recorded from your child's medical notes to see if it has any relationship with RBT readings.

Are there any potential risks in taking part in this study?
Eye pressure measurement by GAT and RBT is carried out routinely in hospitals and at the opticians and is very safe.
As part of the study we will use some eye drops which may cause minor discomfort. Details of what can be expected are listed below;
Proxymetacaine 0.5% is a mild local anaesthetic used to numb the surface of the eye. The drops take about 60 seconds to work and around twenty-five minutes to wear off. Your child should not wear contact lenses for thirty minutes and should avoid situations where he/she might get dust in their eyes as they will not be able to feel it. If unusual symptoms like pain, soreness or blurred vision occur please contact Nicola Sabokbar (details below) or a GP/optometrist as your child may be having an adverse reaction to the drops.

Fluorescein 1% is used to stain the front of the eye to help read the pressure. It can sting a little when first applied and will make the eye look slightly orange, but this will not last long and can be washed away with cold water. If your child wears contact lenses, he/she will be advised not to wear them for at least fifteen minutes to stop the lenses absorbing the stain.
Occasionally it is possible to be allergic to proxymetacaine and fluorescein eye drops, but this would be very unusual.
Your child's eyes will be checked after the procedures to make sure they are fine.

What happens if my child is harmed by the study?
Eye pressure measurement is carried out all the time in hospitals and at the opticians and is very safe. It is very unlikely your child will be harmed by the study but medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS, i.e compensation is only available if negligence occurs.

What are the possible benefits of taking part?
We cannot promise that the study will help your child but the information we get might help us to check the reliability of their eye pressure measurements. Knowing this may benefit other young people who need to have their eye pressures measured by RBT in the future.

Where can I find independent information about taking part in research?
You can contact the NHS Patient Advisory Liaison Service (PALS) at Birmingham Children's Hospital if you would like advice on taking part in research: Email: pals@bch.nhs.uk or telephone: 0121 333 8403

What happens if there is a problem or something goes wrong?
If you have any concerns about the study or want to make a complaint or give feedback about the study, you can contact the researchers named at the end of this information sheet. They will do their best to address your concerns.
If they are unable to resolve the concerns you raise you can contact the secretary to the Aston University Ethics Committee – Mr John Walter – on **j.g.walter@aston.ac.uk** or telephone 0121 204 4869.

Will my child's details be kept confidential?
The eye pressure measurements and data collected from your child's medical records will be stored in paper records in a file in a locked cupboard at Birmingham Children's Hospital and will only be accessible to researchers involved in the study. Access to your child's medical records (to obtain test information) will be undertaken by the research team following your consent. They will follow normal practice in the NHS to ensure your confidentiality. Any data stored electronically on an Aston University laptop will be anonymised and will be password protected. With your permission we will notify your child's GP that he/she is taking part in the study. Your child's data will be kept securely for fifteen years and will then be destroyed.

What will happen to the results of the study?
The results of the research will be published in scientific journals. It will not be possible to identify anyone from the data as all data will be anonymised. You can ask your child's healthcare team or Nicola Sabokbar for information about the results, when the study has finished.

Who is organising and funding the research?
This project is being organised and funded by Aston University. Nicola Sabokbar is the Principal Investigator. She is an optometrist and is undertaking the project as part of her Ophthalmic Doctorate qualification.

Who has reviewed the study?
Before any research goes ahead it has to be checked by an NHS Research Ethics Committees. They make sure that the research is fair. This project has obtained Aston University Governance Approval and has been checked by the R & D team of Birmingham Children's Hospital.

Contact for further information

Chief Investigator: Dr Leon Davies
Telephone: [redacted], email: **l.n.davies@aston.ac.uk**

Researcher: Mrs Nicola Sabokbar
Telephone: [redacted], email: **sabokban@aston.ac.uk**

Clinical Supervisor: Mr Joseph Abbott
Telephone: [redacted], email: **Joseph.Abbott@bch.nhs.uk**

Thank you for reading the information sheet and considering taking part in this study.

PIS Adult (2) date 19th Feb 2016

RBT Study data Case Report Form		DATE
Subject initials		Subject I.D

Inclusion Criteria

	Inclusion criteria	Yes	No
	Subjects must be age 1 month-16, and having eye pressure measurement as part of their care.		
	Subjects have glaucoma a connective tissue disorder or no ocular disease no eye disease (no eye surgery/medication, no high spectacle prescription i.e < +/- 6.00 DS and < -2.00D astigmatism).		
	Subjects must be willing to have Goldman applanation tonometry and rebound tonometry.		

Exclusion Criteria

	Exclusion criteria	Yes	No
	Subjects unwilling to have Goldman Applanation Tonometry and /or rebound tonometry.		
	Subjects with a known allergy to either proxymetacaine or fluorescein eye drops.		
	Pregnancy		
	Premature birth (babies only)		
	High spectacle prescription in eyes with no disease (> +/- 6.00 DS and > -2.00D astigmatism)		

Important- if the yes box is ticked under exclusion criteria, the subject is NOT eligible for the study and should not continue.

RBT Study data		DATE
Subject initials		Subject ID

Data

	Right eye	Left eye
GAT		
RBT on axis		
RBT off axis		
Refractive error		
Axial length		
Corneal curvature		
Recent best corrected visual acuity		
Central corneal thickness		
Glaucoma treatment: medical/surgical/both		
Corneal defects: present/absent		
Strabismus: present/absent		
Nystagmus: present/absent		

Comments: GAT and RBT readings should be taken within 20 minutes if possible.

RBT Study data		DATE
Subject initials		Subject ID

Medical history	
Ocular history	
Age	
Ethnicity	
Sex	

CRF Version 2, dated 25th June 2015

Participant ID Log RBT Study. Principal Investigator Nicola Sabokbar

Assigned ID	Participant name	Participant GP details	Participant contact details

Version 1, dated 25th June 2015

Aston University

NHS
Birmingham Women's and Children's
NHS Foundation Trust

RBT Study results

This study found that:

The rebound tonometer is a good way to measure eye pressure in children with glaucoma and connective tissue disease even when measurements are difficult.

Thank you to all the children who took part in this study

Mrs Nicola Sabokbar
(Principal Investigator)

If you would like to know more please email Nicola:
sabokban@aston.ac.uk