GLYCATED NAIL PROTEIN AS AN INDICATOR

OF LONGTERM GLYCEMIC CONTROL

Dissertation submitted to

THE TAMILNADU
DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment of the regulations
for the award of the degree of

M.D, BIOCHEMISTRY
(BRANCH - XIII)

DEPARTMENT OF BIOCHEMISTRY,
THE GOVERNMENT STANLEY MEDICAL COLLEGE
CHENNAI - 600 001.

THE TAMILNADU
DR.M.G.R MEDICAL UNIVERSITY,
CHENNAI – 600032

APRIL 2015

CERTIFICATE

This is to certify that the dissertation entitled, **"GLYCATED NAIL PROTEIN AS AN INDICATOR OF LONGTERM GLYCEMIC CONTROL,"** is the bonafide record of the original work done by the candidate, **Dr.CACTUS LILY JEYARAJ**, in the Department of Biochemistry, Government Stanley Medical College and Hospital, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of the degree of M.D. (Biochemistry) Branch - XIII during the academic period of 2012 – 2015.

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DECLARATION

I**, Dr. CACTUS LILY JEYARAJ**, solemnly declare that this dissertation, titled, "**GLYCATED NAIL PROTEIN AS AN INDICATOR OF LONG TERM GLYCEMIC CONTROL,"** is a bonafide work done by me in the Department of Biochemistry, Stanley Medical College and Hospital, Chennai, under the guidance of Professor **Dr.R.LALITHA, M.D. D.A.,** Government Stanley Medical College and Hospital, Chennai - 600 001. This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of the degree of M.D. (Biochemistry), Branch XIII, examinations to be held in April 2015.

Place: Chennai

Date: **Dr. CACTUS LILY JEYARAJ**

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ABBREVIATIONS

WHO	–	World Health Organization
DM	–	Diabetes Mellitus
BMI	–	Body Mass Index
Hb	–	Hemoglobin
HbA1C	–	Hemoglobin A1C
ADA	–	American Diabetes Association
NIDDM	–	Non –insulin Dependent Diabetes mellitus
BG	–	Blood Glucose
FBS	–	Fasting Blood Glucose
MBG	–	Mean Blood Glucose
GTT	–	Glucose Tolerance Test
HPLC	–	High Pressure Liquid Chromatography
NGSP	–	National Glyco hemoglobin Standardization Programme
NBT	–	Nitro Blue Tetrazolium
TGL	–	Triglycerides
HDL	–	High Density Lipoprotein
LDL	–	Low Density Lipo proteins
ROC Curve	–	Receiver Operating Characteristic curve


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ABSTRACT:

GLYCATED NAIL PROTEIN AS AN INDICATOR OF LONG TERM GLYCEMIC CONTROL

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Key words: Nail fructosamine, glycation, long term control

A study was conducted in Government Stanley Medical College and Hospital, in the Department of Biochemistry, titled glycated nail protein as an indicator of long term glycemic control .

A total of 70 individuals, 35 known diabetic patients and 35 healthy individuals were enrolled for the study. The patients were longitudinally followed up for three months to assess their glycemic status.

The aim was to establish whether the nail fructosamine could be used to determine the glycemic status of the diabetic patients over the preceding three months which is the turnover time of the nail from root to the free edge. The

Hemoglobin A1C estimation in the same patients at a same time was used to confirm the changes in the patient's glycemic status and compared with nail fructosamine. A linear relationship was established after analysis of the results. (p value 0.009 . A value of less than 0.5 is significant.

There was also a significant difference of nail fructosamine between cases and controls (p value 0.09) .The mean value of controls (2.18µmol/g equivalents of nail) lie in the physiological range (2.0-2.5µmol/g)as reported by Goldsmith et al in 1985.

The patients values determined initially and finally were above the physiological range (2.68 and 3.1µmol/g)

The fasting blood glucose and serum fructosamine were also measured for assessing the blood glucose levels. These parameters on comparison with nail fructosamine showed linear relationship.

No interference was done in the treatment schedule of the patients.

The risk factors like positive family history, blood pressure, BMI, lipid profile were also analyzed. Results showed only moderate increase in risk factors among patients than in the controls.

To conclude the nail fructosamine assay could be standardized for testing the levels of blood glucose over the previous three month's duration.

The reference range in south Indian population needs to be established.

The interference like drugs and other dietary substances which might react with nitrobluetetrazolium need to be studied and the procedure can be used as routine laboratory investigations to assess the blood glucose levels on long term basis. The method is cheaper, easier to perform and sample collection and preservation is easier (nail clippings form finger nails) and noninvasive.

Pre analytical variation of the analyte as observed with other analytes is practically nil.

Cost of testing is less compared to other indices. Precision of the assay is good.CV Calculated was 7.8%

INTRODUCTION

DIABETES THE CLINICAL SCENARIO:

Diabetes mellitus has become a worldwide epidemic and has to be identified and treated as early as possible to avoid micro- vascular and macro -vascular complications. Diabetes is a universal health problem which affects millions of people causing high morbidity and mortality.

WORLD WIDE STATISTICS:

As per WHO report, prevalence of diabetes is as shown below :

YEAR	PREVALANCE
1985	100 million
1995	135 million
2010	221 million

TABLE – 1 : DIABETES PREVALENCE

DIABETES IN SOUTH ASIAN COUNTRIES:

Bangladesh, Bhutan, India, Pakistan, Maldives, Nepal and Sri Lanka contribute to nearly 25% of the diabetic population living throughout the world. (1)

S. No	Country	Urban : Rural ratio
1.	INDIA	1.2 – 2.4
2.	Bangladesh	3.5
3.	Pakistan	1.4
4.	Sri Lanka	1.9
5.	Nepal	1.1 -5.8

TABLE – 2 : DIABETES IN SOUTH ASIA

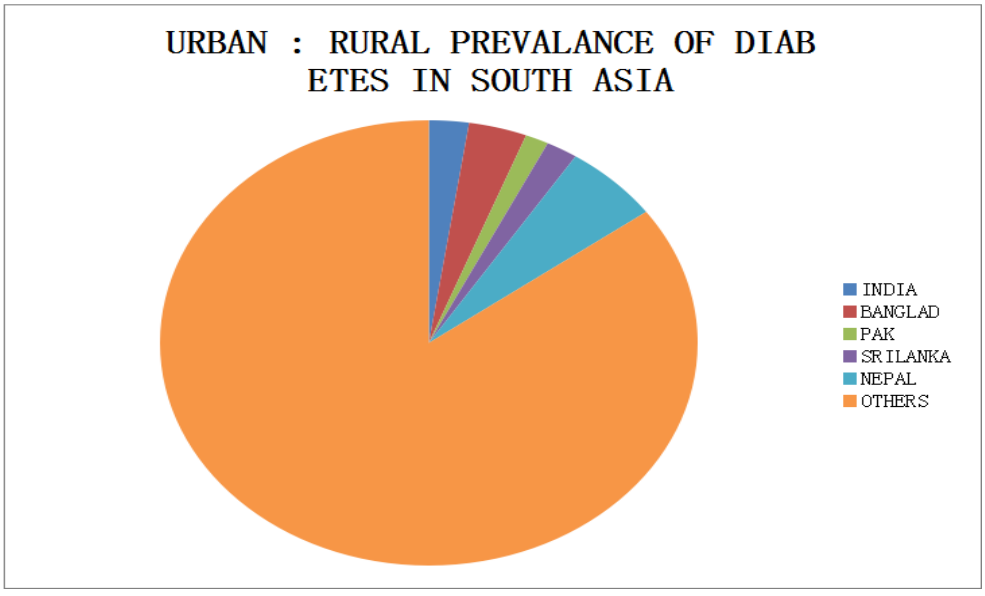


Fig.1 : THE TREND OF URBAN TO RURAL PREVALENCE OF DIABETES

The life-style of rural population has been changing and the fast – food craze has landed up in the rising trend of obesity and diabetes in India.

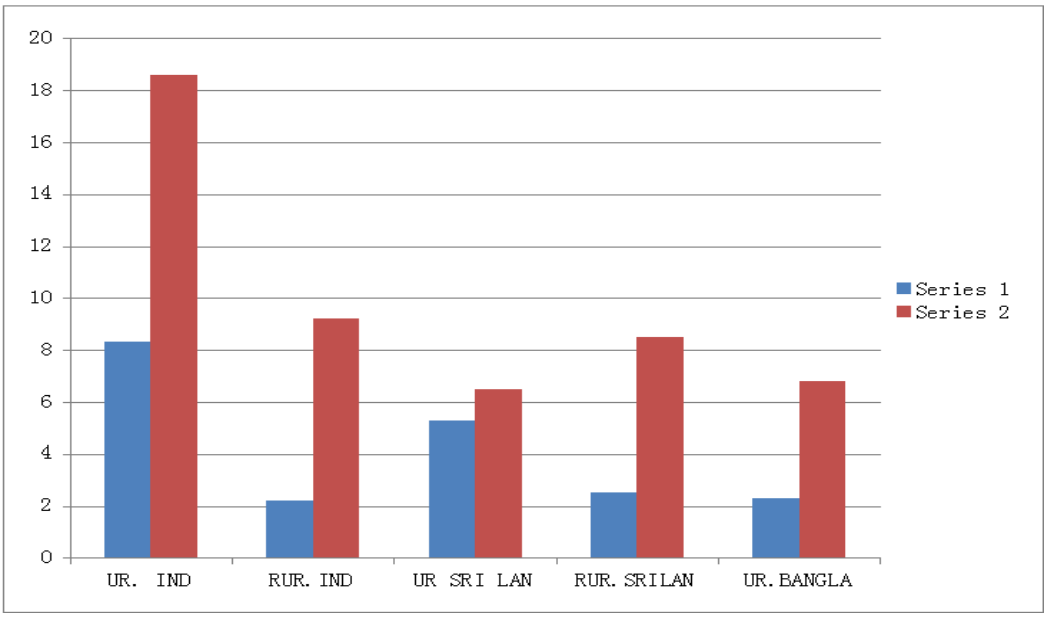


Fig 2. PREVALANCE TRENDS IN SOUTH ASIAN COUNTRIES

Following the western culture in Indian climatic conditions and technological advances that deprive us of our physical exercise make us succumb to the giant threatening the nations despite rich or poor.

The cost of Diabetes management places burden on the shoulders of the country's economy. (2)

The national health bodies and their projects are lagging behind in coping up with the disease progress.

The identified risk factors in our population according to Jayawardane et al. in 2012 (3) are shown in the following table:

Table: Pooled odds ratios for diabetes risk factors in South Asians

Risk factor	Pooled OR (inverse variance)	95% confidence limit
Family history	2.75	2.11, 3.58
Age	1.37	1.25, 1.50
Male gender	1.21	0.97, 1.50
SBP	1.49	0.94, 2.36
DBP	1.06	1.04, 1.07
BMI	1.17	1.12, 1.22
Waist:hip ratio	1.36	1.22, 1.52

BMI, body mass index, DBP, diastolic blood pressure; OR, odds ratio; SBP, systolic blood pressure.

TABLE - 3 : RISK FACTORS FOR DIABETES IDENTIFIED IN SOUTH ASIAN COUNTRIES

There appears to be an increased incidence of DM at a younger age in our population and the risk is increased with even lower BMI.

We require cost effective treatments and investigations to reach out to the rural population showing an increase in prevalence. The need to educate people about early symptoms and routine health checks are also vital to curtail the disease from engulfing the whole nation.

In this study an attempt is made to establish a newer cost effective, simple, precise and noninvasive method of testing for long term glycemic control by estimating the glycated nail protein (ie.) fructosamine present in the keratin of finger nails.(4)

The cost of testing for blood glucose is three times higher and HbA1C is 15 to 40 times higher than the cost for nail fructosamine testing.

The aim of the study is to establish whether finger nail fructosamine could be used to determine the long term glycemic status of an individual over the previous three months. Three months makes sense that the duration for growth of finger nail from nail matrix to the free distal margin takes on an average of three months.

The objectives are to ensure the validity of this test by comparing the individual's glycemic status using the existing standard glycemic index, HbA1C and also with serum fructosamine.

REVIEW OF LITERATURE

DIABETES MELLITUS

HISTORY OF DIABETES MELLITUS

The earliest description of diabetes is evidenced in Ebers Papyrus the literature from 1550 BC. by Imhotep (5) a high priest, man of medicine who described polyuria.

Arateus of capppadocia (150 AD) in his book acute and chronic diseases coined the term “diabetes” meaning “siphon” which implies the “liquefaction” of flesh and bones into urine (6).

Susruta in 400BC described the syndrome of Diabetes having a honeyed urine(7).

Fredrick Banting, John McLeod Charles Best and JR. Collip, during 1921were the discoverers of insulin and showed that pancreatic extract from dogs lowered blood glucose levels .(8)

1922 - (Leonard Thompson 14 years of age at Toronto general hospital in Canada) received purified pancreatic extract for correction of hyperglycemia and metabolic acidosis .(FIRST HUMAN INSULIN)

1925 – 66 - Newer synthetic insulins were developed. (9)

1955 – Sanger and collegues – succeeded in amino acid sequencing of insulin and defined its structure. (10)

1982 – recombinant human insulins .(11)

CLASSIFICATION OF DIABETES MELLITUS : A CENTURY SINCE 1880

1. Type 1 IDDM (Insulin dependent diabetes mellitus)
2. Type 2 NIDDM (Non- insulin dependent diabetes mellitus)
3. Gestational diabetes mellitus
4. Impaired Glucose tolerance
5. Impaired fasting glucose

Secondary diabetes – associated with pancreatic diseases,removal endocrinopathy and genetic syndromes.(12)

Classification: WHO 1985: (13)

1) IDDM
2) NIDDM
3) Malnutrition related diabetes mellitus
    a) fibro- calculous pancreatic diabetes
    b) Protein deficient pancreatic diabetes
4) Gestational diabetes mellitus

5) Other types associated with diseases like glucaganoma, thyrotoxicosis, drug induced eg. Diazoxide, phenytoin, furosemide, insulinopathies etc.

WHO CRITERIA for Glucose tolerance test interpretation using 75 g glucose load:

Type	2-h OGTT result, mmol/L (mg/dL)	
	0 h	2 h
Impaired fasting glucose[b]	>6.1 (110) to <7.0 (126)	<7.8 (140)
Impaired glucose tolerance[c]	<7.0 (126)	>7.8 (140) to <11.1 (200)
Diabetes[d]	>7.0 (126)	>11.1 (200)

TABLE : 4 - WHO CRITERIA TO DIAGNOSE DIABETES

Any results obtained within the diagnostic limits should be repeated on another day before initiating any form of treatment.

ADA Criteria:2010 (14)

Fasting Plasma Glucose	≥ 126 mg/dL
Casual	≥ 200mg/dL
2 hours (post 75gGlucose load	≥ 200 mg/dL
HbA1C	≥ 6.5%

In the criteria of WHO expert committee 1997,HbA1C is not included.

Casual – Any time of the day without relationship to previous food intake.

Pathogenesis of NIDDM:

Two defects are reported, (15)

1. Insulin resistance
2. Beta cell dysfunction of pancreatic tissue.

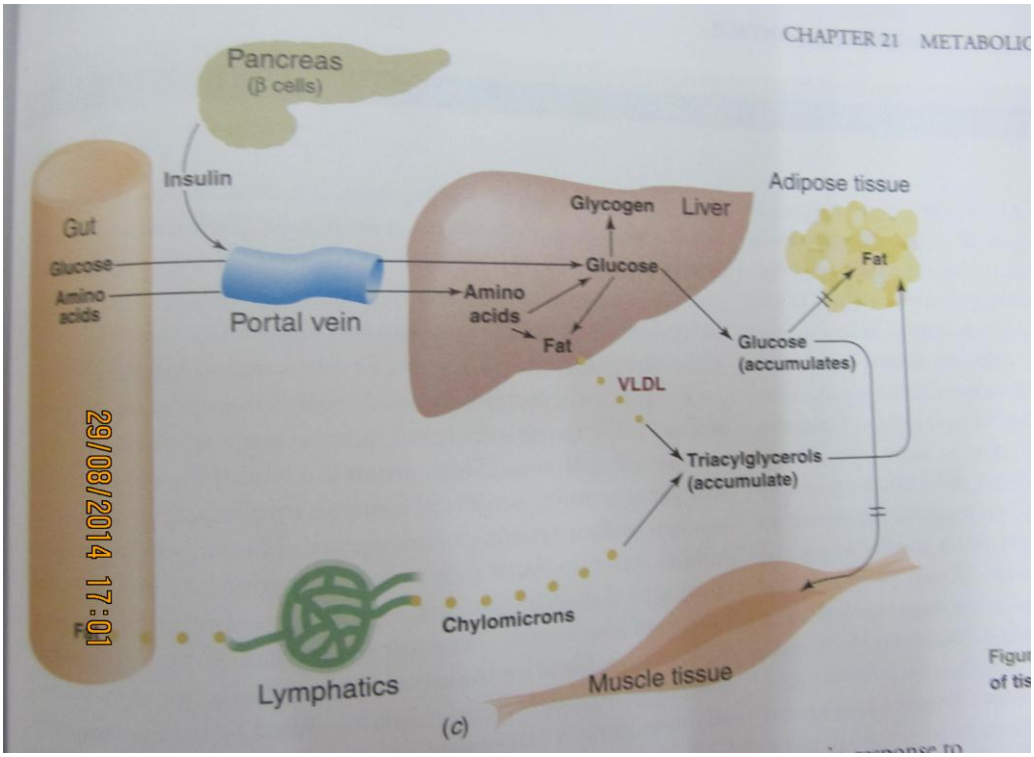
Insulin resistance is defined as a decreased biological response to normal concentration of circulating insulin. **The primary cause is obesity and the quantity of body fat is proportional to insulin resistance**. (16) TNF α and resistin by adipose tissue oppose the action of insulin. A decrease in levels of adiponectin also contributes to insulin resistance.

Beta cell dysfunction develops secondarily and there is a loss of glucose induced insulin release. This is termed as **selective glucose unresponsiveness.**

n inverse relationship exists with degree of physical activity and development of the disease.

Genetic factors also contribute to the disease.

FIG.3 - PATHOGENESIS OF NIDDM



EPIDEMIOLOGY:

The reports of WHO suggests the prevalence of 100 million in 1985,151 million in 2000, 221 million in 2010.

The explosive increase of non- insulin dependent diabetes mellitus occurring due to a sedentary life style places a burden on the economic status of the country as it would cost more for diagnosis, management and supportive care for the micro vascular and macro vascular complications of the disease.

Historical aspects:

Pre insulin era:

Mathew Dobson in 1776 – boiled urine of diabetics till dry and found its resemblance to brown sugar .(17)

John Rollo, in 1797 - reported the presence of increased sugar in the blood of diabetics.

Wollaston 1828 - attempted to measure blood sugar, but did not succeed.

Chevreuil in 1914 -reported the sugar in the blood resembled dextrose or glucose .(18)

Specific methods to measure blood glucose as reducing substance developed later.

TOOLS IN DIAGNOSING DIABETES: URINE GLUCOSE, BLOOD GLUCOSE

The **Benedicts urine test** used by S.R. Benedict was time consuming and caused bad odour. The next step was **clini-test tablets** which gave a crude measure of glucose control during 1950s.

Self monitoring of Blood Glucose SMBG was introduced in 1970 with glucose oxidants impregnated strips. They are a boon to diabetic patients and treating physician to achieve a good glycemic control. Other advancements are gluco-watch based on iontophoresis which measures glucose in subcutaneous fluid or a subcutaneous needle to monitor blood glucose continuously(19).

NAIL AND KERATIN

History

Structure and functions

Types

Composition

Glycation

Keratin belongs to the class of fibrous proteins. Fibrous proteins are classified into three classes.

1. Keratins which form protective covering of the skin, hair and nails, hooves, horns, scales beaks, quills feathers, fur and wool. Actin and myosin of muscle and fibrinogen which takes part in coagulation are other fibrous proteins.

2. Silk and insect fibers.

3. Collagen and hides which give structural support and strength.

A fibrous protein has polypeptide chains arranged in long strands or sheets. The Cα-N bond axis (φ) and Cα-C axis(ψ) are unchanging and recur over long stretches of the chain

Structure:

Alpha keratins are made up of alpha helices as suggested by W.T.Astbury in1930, wound around in a right handed coil like Aubusson tapestry .(thread-coiled coil) (20)

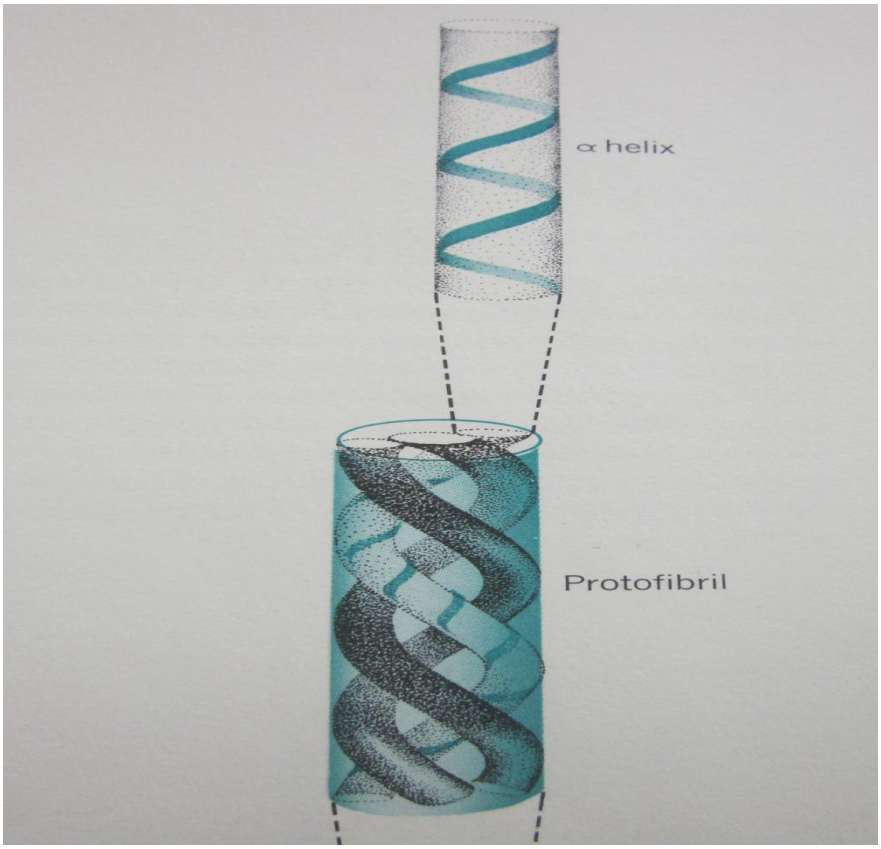


Fig. 4 : BASIC UNIT OF KERATIN

THE ALPHA KERATIN:

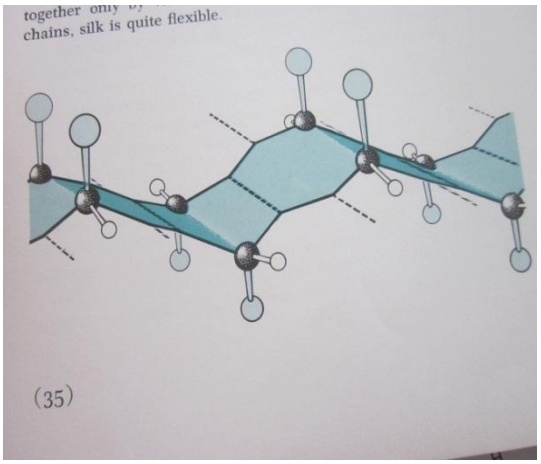


FIG . 5 : ALPHA HELIX **FIG. 6 : THE BETA SHEET**

Proto fibrils (20A°) are the basic units that form keratin. One protofibril is made up of three right handed alpha helices arranged in a left handed coil. Nine protofibrils are bundled together surrounding two more of axially arranged protofibrils to form a micro fibril (80A°) .This forms a eleven stranded cable like structure. The super twist is left handed and the places where the three helices contact contain hydrophobic aminoacids. R groups of aminoacids in the helices are found in interlocking pattern. Hundreds of microfibrils which are embedded in sulphur -rich protein matrix are cemented to form irregular bundles called macro fibrils. (21)

The helices are crosslinked with disulfide bonds of cysteine residues.The hydrogen bonds also strengthen the helices but are weak.

The keratins are classified as soft (skin) and hard keratins (horn, nail, hooves) by their sulfur content.

Beta keratin:

Classical examples are wool and feathers where polypeptide chains are arranged in axis parallel to the fiber axis. Neighbouring chains are linked by hydrogen bonds and run in opposite directions and forms sheet like structure. (22)

Composition of the nail:

The keratins of nail belong to either low sulfur content group of molecular weight 40 – 60 kDa which constitutes 20% or high sulfur group of keratins (10 – 25 kDa) about 80% and a high glycine- tyrosine matrix proteins(6-9 k Da) (23). The fibrils are seen embedded in the matrix. Regional differences of keratin expression within the nail is also noted. The main lipid of the nail is cholesterol. Fat content is 0.1 – 1% and water content is 7 – 12%, calcium is 1% of weight, sodium, magnesium and phosphorus are increased in cirrhotics. Iron and copper are increased in children. Alcohol can permeate and hydrophobic molecules and steroids can permeate into the distal nail plate.(24)

Over 30 types of high sulfur proteins are identified in the human nail.

TYPES OF KERATIN AND ITS DISTRIBUTION:

More than twenty types are known.(25)

Alpha	Beta	Occurrence
Keratin1,2	9,10	Cornea , keratinocytes
3	12	Cornea
4	13	stratified epithelium
5	14,15	stratified epithelium
6	16,17	Squamous epithelium
7	19	ductal epithelium
8	18, 20	simple epithelium

TABLE - 5 : TYPES OF KERATIN

Keratins expressed in **the nail:**

TABLE : 6 - KERATIN EXPRESSION IN NAIL

S.No	Type	Structure expressed
1	K5/K14	Nail bed , germinative matrix
2	K6/K16	Nailbed
3	K1/K10	Proximal nail fold germinative matrix

Trichocyte keratins expressed in the nail are HaKa1-4,Ha Kb1-4 and HaX and HbX.(26)

GLYCATION:

Glucose binds to epidermal proteins non-enzymatically as seen in serum proteins and hemoglobin but at a slower and constant rate proportional to the glucose concentration in the extracellular fluid.

"Glucose binding to keratin and proteins of the insoluble stratum corneum fraction appeared to occur at practically the same rate, and it is a first-order reaction with regard to the glucose concentration. Water-soluble proteins of the stratum corneum undergo non-enzymatic glycation preferentially (on average, 83.4% of the total amount of glycated proteins

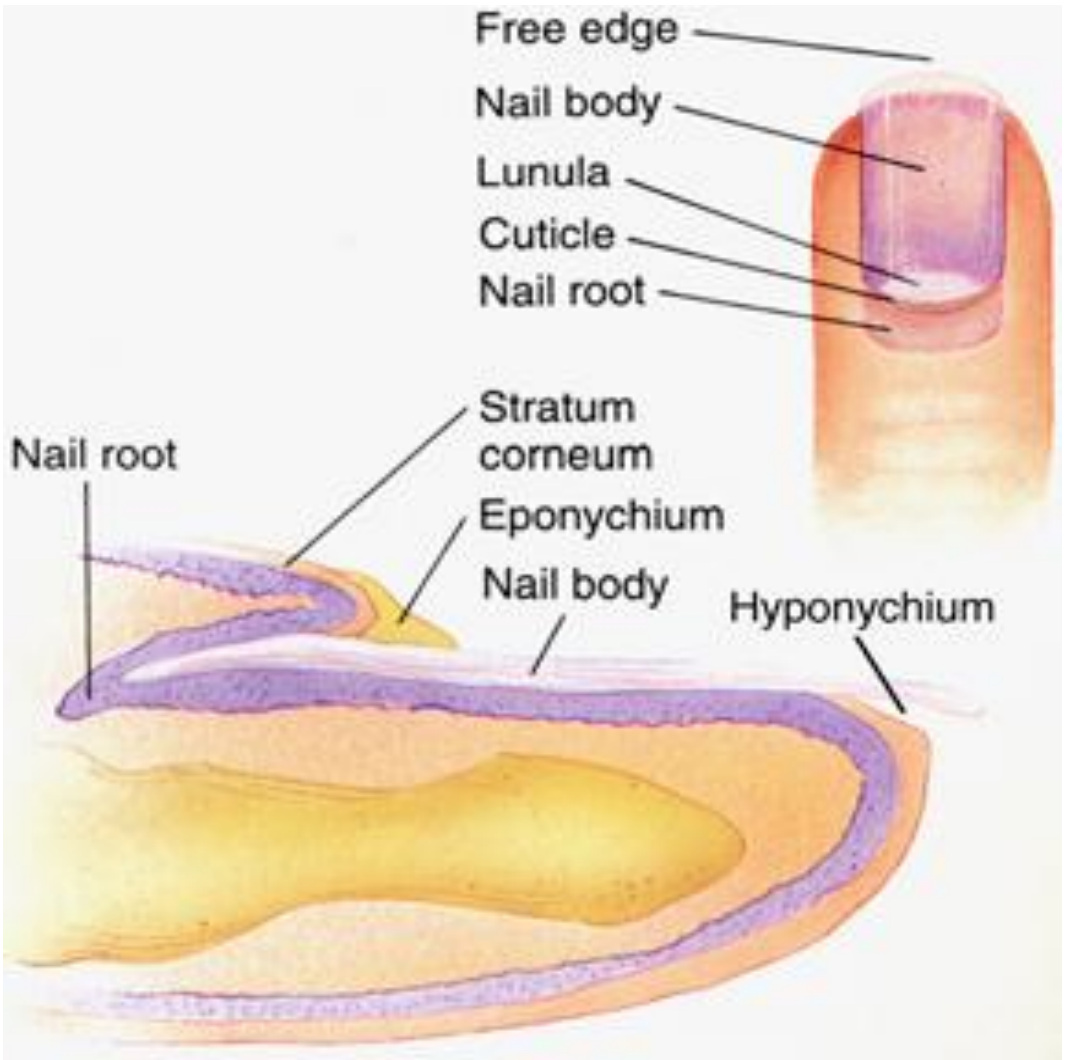
is present in the soluble fraction), regardless of the initial content of glycated proteins in the sample."(Marova et al 1995)(27)

THE HUMAN NAIL:

Nail structure:

Nail anatomy is the same in fingernails & toenails

FIG - 7 : STRUCTURE OF HUMAN FINGER NAIL



It comprises of

Nail matrix

nail plate and

cuticular system

Nail bed

Nailfolds

Lunula

The nail plate is made up of hard keratin and is of horny and transluscent nature.

It varies in thickness between **0.5 and 0.75 mm.**

Histology:

Three layers are identified

Thin dorsal lamina

Thick intermediate layer

Ventral layer.

The ventral layer is about one-fifth of the nail thickness and is derived from the nail bed and the rest from the nail matrix.

Microscopic appearance:

The nail plate shows closely packed flattened squamous cells in close opposition because of tortuous and interlocking plasma membranes (28).The soft tissue where the nail overlies is called the nailbed.

It consists of

1. A vascular matrix contiguous with the periosteum of the distal phalanx.

2. Two or three celled layer of epidermis (living layer of cell) above which dead cells of the ventral nail plate lies. As the cells differentiate, they are incorporated into the ventral surface of the nail plate and move distally with this layer.

Physiologic factors affecting nail growth rate:

Finger nails grow at a rate of 0.1mm per day and toe nails at one third of this rate. (29)

1. Young age, pregnancy ,male gender - increased growth rate

2 . Lactation, old age, female gender - decreased growth rate

THE HEMOGLOBIN AND ITS GLYCATION

Structure and function:

Hemoglobin is exclusively found in the red blood cells and transports oxygen to the tissues

Composition:

Hemoglobin consists of two alpha chains and two beta chains encasing a heme moiety within the core of each chain.

Types:

Normal hemoglobins:

TABLE – 7 : NORMAL HEMOGLOBIN

Name	Composition	% distribution
Adult A1	Aαßß	Major form97%
AdultA2	Aαδδ	Minor form < 3%
Foetal form-HbF	ααɤɤ	Major form in foetal life

Mutant forms:

They are significant to a clinical chemist because they interfere in the HbA1C assay.

Various minor components have been identified.

Commonly encountered ones are the following:

Name	Normal aminoacid–position-charge	Replaced by charge
HbS (Sickle)	Glutamic acid – ß 6 -negative	Valine positive
HbC	Glutamic acid – ß 6 -negative	Lysine positive
HbE	Glutamic acid – ß 26 -negative	Lysine positive

TABLE - 8 : HEMOGLOBIN VARIANTS

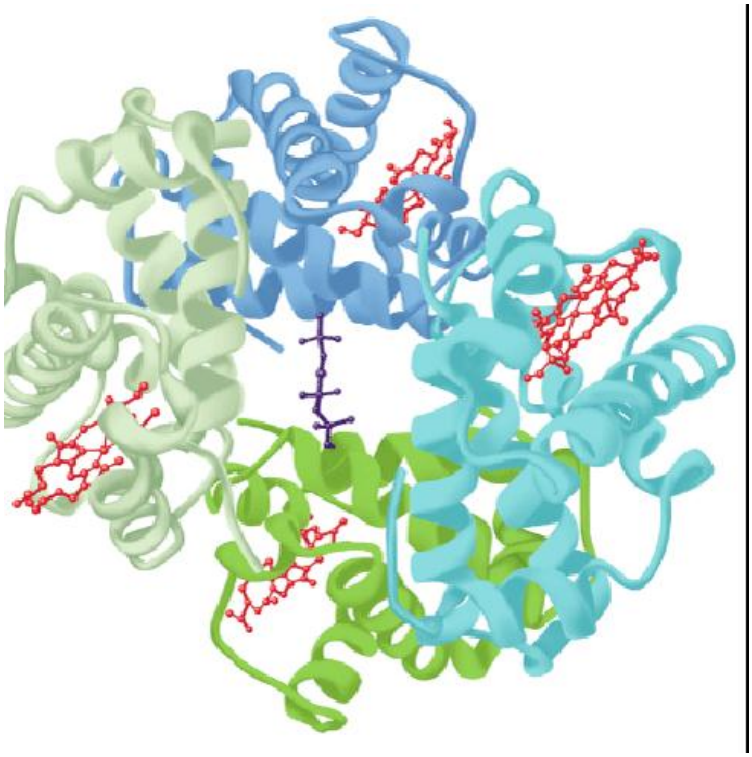


Fig. 8 STRUCTURE OF HEMOGLOBIN

The structure of heme is designed in such a way that the methene bonds resonate and it has a characteristic absorption spectrum.

All hemoglobin derivatives have characteristic absorption spectra.

The variant hemoglobins do not differ except for methemoglobin which also differs from the spectrum of HbM.(30)

This property is made use of in estimating the total hemoglobin in immuno-inhibition assay for HbA1C estimation in this study.

GLYCATION IN HEMOGLOBIN:

The non- enzymatic addition of a sugar residue to the amino group of proteins is called glycation.

The IUPAC terminology used is neo-glycoprotein. The term glycation is preferred and widely used. (31)

The normal hemoglobin consists of HbA1 - 97%, HbA2- 2.5%and HBF<0.5%.

Chromatographic separation of HbA identifies several minor hemoglobins.

HbA1a1- fructose 1,6 di phosphate to N terminal valine of the ß chain.

HbA1a2 - glucose 6 phosphate attached to N terminal valine of the ß Chain

HbA1b - pyruvic acid attached to N terminal valine of the ß chain

HbA1C - glucose attached to N terminal valine.

The rate of glycation is directly proportional to the glucose concentration in the blood and is unaffected by daily fluctuations.

Hence it is considered as a tool for determining the long term glycemic control.

HEMOGLOBIN A1C - STRUCTURE

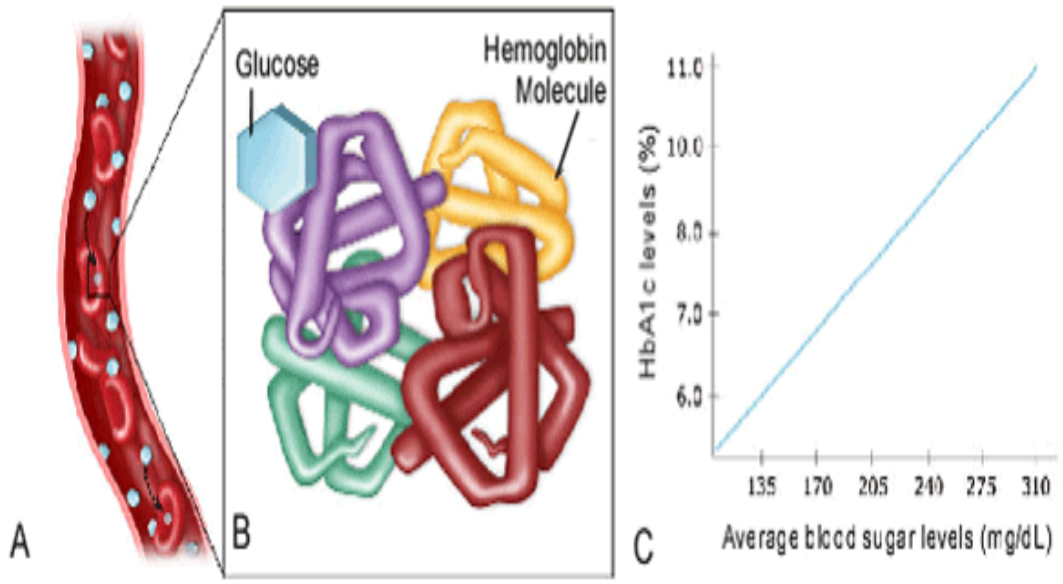


Fig - 9: A. Blood vessel B. Glycated hemoglobin

C. HbA1C versus Blood Glucose

History of HbA1C

1958 – Huilman and Mayering separated HbA1c using chromatographic columns.

Samuel Rahbar etal – found an increase in proportion of HbA1c is seen in diabetes(32)

1975 – Bunn and co-workers described the reactions occurring at the molecular level

1976 – Antony Cerami Ronald, Koenig and coworkers proposed it for monitoring the degree of control of glycemia.

Methods for detecting HbA1C:

1. High Pressure Liquid chromatography (HPLC)
2. Immuno assays
3. Enzymatic methods
4. Capillary electrophoresis.
5. Boronate affinity chromatography
6. Colorimetric techniques.

Point of care devices use the boronate affinity or immuno - assays for testing HbA1C.

THE GLYCEMIC INDICES: (33)

Short term control :

1. Plasma glucose (hours)
2. Fructosamine serum (1 -3 weeks)
3. Glycated albumin serum ((1-2weeks)
4. 1,5- anhydro glucitol serum (1 -14 days)
5. Long term control:(6 – 12 weeks)

HbA1C and A1C Derived Average Glucose (calculated parameter)

REACTION OF GLUCOSE WITH HEMOGLOBIN A1C:

The glucose transporters in the RBC membrane allows glucose entry into the cell.

And they when present in excess bind to N terminal valine and epsilon amino group of the protein portion of the hemoglobin.

The binding is initially reversible (PRE HbA1C- the unstable aldimine form) (34)and glucose can re- enter into the cytosol if high blood glucose levels are not persistent.

But whenever the duration of this temporary association is prolonged, there occurs a permanent modification of the binding called AMADORI rearrangement. (35)

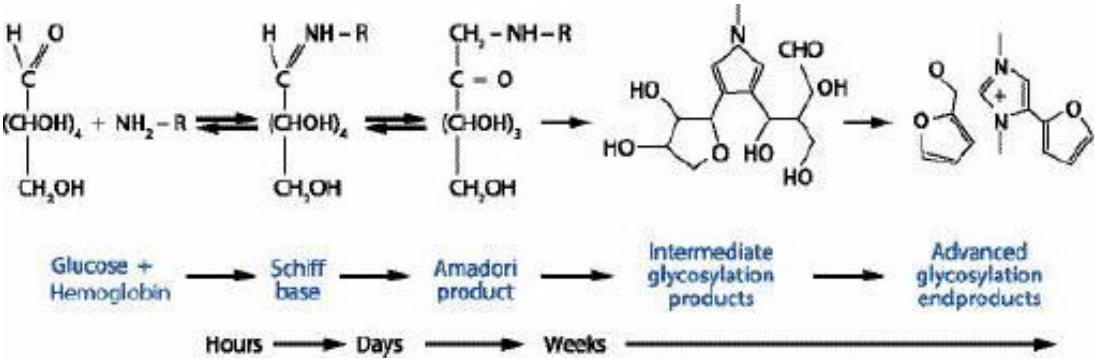


FIG.10 - GLYCATION AT THE MOLECULAR LEVEL.

The more the excess in blood glucose, the more is the glycation . The glycated product thus formed is called GLYCATED HEMOGLOBIN - HbA1C.

This is the reason why the percentage of glycated hemoglobin is more in a patient with diabetes. The glycation degradation products damage the membranes and cause organ damage.

SIGNIFICANCE OF HbA1C:

The significance of HbA1C is that it gives an average estimate of blood glucose in the individual and it tells retrospectively the same, for a period of 6-12 weeks ie the average life span of RBC.

This is the concept behind the determination of HbA1C as an indicator of long term glycemic control in diabetic patients.

Glycation occurs in normal population and <5% of hemoglobin is expected to undergo glycation in euglycemia.

HbA1C as the glycemic index:

Advantages:

1. A random, non-fasting venous blood sample with anticoagulant can be used for the assay.
2. Minimal sample volume obtained directly from a finger prick is adequate in point of care (POC) testing devices.
3. Results are also faster in POC devices (8 minutes) and routine procedures followed for testing.(15 – 30 minutes).
4. The assay has been standardized by NGSP . (36)

Disadvantages:

1. Results are not reliable in individuals with hemoglobin variants as they interfere in the routinely used HPLC methods.

2. Anemias where red cell life span is affected.

3. Needs sophisticated and costly equipments and hence the high cost of testing.

GLYCATION OF PROTEINS:

Apart from glycation of hemoglobin, the proteins where glycation is known to occur is collagen , tubulin, ocular lens protein, peripheral nerve protein, tendon protein, arterial proteins, elastin in lung anti-thrombin III, erythrocyte membrane proteins ,fibrinogen, lipoproteins, ribonuclease A, platelet protein, urinary amino acids and peptides.(37)

The glycation process is typically non enzymatic and it differs from the enzymatic process which occurs post- translationally during protein synthesis.

The same product occurs in all proteins of plasma, cell membrane and extracellular matrix and is collectively termed as fructosamine in general and glycated albumin when it is spoken of in albumin.(38)

Fructosamine assays available are

1. Chemical methods:

They measure nonspecifically all glycated lysine residues.

A) Nirto blue tetrazolium method

B) Thio barbituric acid method

C) Phenyl hydrazine method

D) Furosine method

2. Chromatography:

Boronate affinity chromatography

3. Immuno Assays:

Immuno turbidimetry using specific antibodies to glycated albumin

4. Enzymatic method:

Using endo-peptidases and lysyl oxidase by photometric techniques.(39)

Advantages:

Serum fructosamine is a cheaper method and shows short term control of glycemia over weeks.

The assay can be performed in cases of hemoglobin variants when HbA1C is not reliable.

It can be of use in gestational diabetes.

Disadvantages:

The assay standardization and confounding factors limit its usefulness.(40)

METHODOLOGY

INTRODUCTION:

Recent studies(41) showed that fructosamine in nail estimation can be used as a measure of long term control of blood sugar in diabetic patients. In finger nails it gives an estimation of blood glucose levels over the previous three months. Nail clippings are taken for testing.

For Hb A1C the standard methods in use are HPLC method , immuno-turbidimetry and enzymatic methods, all of which are expensive whereas nail fructosamine by Nitroblue tetrazolium method is cost effective.

AIM

TO TEST FOR LONG TERM GLYCEMIC CONTROL USING A NEWER PARAMETER- GLYCATED NAIL PROTEIN (FRUCTOSAMINE) IN FINGER NAILS.

OBJECTIVES:

- To prove that fructosamine levels in nail protein (finger nails) by Nitro Blue Tetrazolium method can be a useful indicator of long term glycemic control (over the past 3 to 5 months) in diabetic patients

- To analyze the efficacy of the method by comparing it with glycated hemoglobin (HbA1C) by immuno - turbidimetric method

- To compare with serum fructosamine levels by NBT method

STUDY DESIGN:

Place of study : Stanley Medical College and Hospital,Chennai.

Duration of study : 6 months April 2014-September2014

Type of Study : Longitudinal study.

Sample size and mode of sampling: Simple random sampling

Cases: 30 diabetic patients

Controls:30 healthy non-diabetic individuals.

Inclusion Criteria:

Type II Diabetic patients (ADA Criteria FBS > 126 mg%) .

The patients included are on

Oral hypoglycemic agents alone or

On Insulin and Oral hypoglycemic agents

Exclusion Criteria:

Diabetic patients with

Nephropathy

Retinopathy

Persistent hyperproteinemia due to any cause

Those with diseased fingernails

Artificial coloring on nails eg. Mehenti or nail polish

Materials required:

1. Hb A1 C kit for immunoturbidimetric method
2. For nail Fructosamine and serum fructosamine assay:
   a. Nitro Blue Tetrazolium reagent – colorimetric method
   b. Fructosamine calibrator

Specimen collection and processing:

1. Nail clippings about 10 mg.(Two samples – initial and final)
2. Venous blood with anticoagulant 3 ml. (HbA1C) collected in potassium EDTA containing vacutainer.

   (Two samples - initial and final)
3. Three ml of whole blood for separation of serum. (fructosamine)

Once in three weeks.

Sample is collected in clot activator containing disposable tubes and allowed to stand for one to two hours as required and serum collected and stored in -20° in a deep freezer.

Methods:

1. Nail fructosamine assay: Endpoint - Bakan et al.(42)

* Nail clippings of 10 mgs are weighed after standardizing the balance
* Cut into pieces of about 2 – 3 mm
* 1 ml of NBT reagent added
* Incubated at 37°C for 1 hour
* Readings taken at 530 nm.
* The values are compared with absorbance of fructosamine standard and results are calculated.

2. Serum fructosamine assay:Kinetic (43)

* To the serum NBT reagent is added and rate is assayed.

* The rate of formation of formazan compound is proportional to the amount of fructosamine present in the serum.

* Because of good precision this assay is preferred.

3. HbA1C: (Immuno turbidimetric assay)

* This is an NGSP certified method and interferences by variant * hemoglobins are less. Hence this method is chosen for assay of HbA1C.

* Both initial and final HbA1C were done for every patient at the beginning and end of the study respectively, and at the same time nail clippings are taken.

* Interval allowed between the two samples varied between 8 – 12 weeks as feasible with patient compliance.

Ethical considerations

The integrity, safety and identity of participants in this study were preserved as per the recommended guide lines by the Institute's Ethical Committee.

The study was approved by the Institute's Ethical Committee, The Government Stanley Medical College and Hospital, Chennai -600001.

Consent:

A written, informed consent was obtained from the patients who were willing for follow up regularly and to participate in this study.

PATIENT SELECTION AND PARAMETERS :

Selection of patients for the study was done from the patients attending Diabetology Outpatient Department of Government Stanley Medical College and Hospital after getting approval from the Institute's Ethical Committee. NIDDM was chosen for the study because acute variations in the blood glucose level is less rather than in IDDM. The study was observational and the selected patients were followed up longitudinally for three months for determination of their glycemic status either weekly or biweekly follow-ups .The indices determined to assess the glycemic status in this study are

1. Fasting blood glucose –mean
2. Serum Fructosamine Mean
3. HbA1C initial and final and average estimated glucose (derived value from HbA1C - (MBG)

The three indices are correlated with nail fructosamine of the same individual to evaluate this promising cost effective glycemic index and its usefulness in longterm glycemic control.

Control group:

Healthy age and sex matched individuals with or without a positive family history were selected for the study. The same parameters were performed for all controls after obtaining informed and written consent.

Assessment of Nail growth rate:

Also the individual nail growth rate was assessed for one month for the population studied to verify the nail turn over time.The nail turnover time was found to be 0.1mm per day on an average.The measured values varied between 3-5mm in a month among the one hundred and four individuals evaluated.

Patient ID and documentation:

The patients selected were issued Identification number as and when they were registered for the study.

They were evaluated based on the proforma and clinically assessed whether they were fitting into the criteria for this study.

The observations were recorded and the data analysed using spss version16.

A physical status examination was also conducted.

Confirmation of Diabetes mellitus :

Fasting Blood Glucose (FBG)>126mg% (44)

Testing for exclusion criteria:

1. Urinary Protein Creatinine Ratio

2. Serum Protein and Albumin.

Urine Protein creatinine ratio:

Specimen collection and processing:

Morning void fresh urine was collected in clean plastic containers and examined within one hour of collection.

The urine collected was kept in the refrigerator if any delay is expected in testing of the sample.

Samples were centrifuged if found turbid .

Method:

Sulphosalicylic acid method Kingsbury's method:(45)

Instrument used :

Semi-automated analyser (Merck Microlab 300)

Principle:

The proteins present in the urine reacts with sulpho-salicylic acid to form turbidity

Reference Interval: <80mg/day at rest.

Upto 150 mg/ day after strenuous exercise.

Estimation of Urinary Creatinine:

Method:

JAFFE'S KINETIC METHOD(46)

Reference interval:

1-1.5 mg/day

Urine Protein Creatinine Ratio: (47)

Reference interval :

0.02 – 0.2

> 0.2 gross proteinuria(after confirmatory test)

Estimation of serum proteins:(48)

Specimen preparation:

Serum separated from whole blood centrifuged at 2000 rpm for 10 minutes and tested on the day of collection

METHOD:

BIURET METHOD -Endpoint.

Aim:

To estimate total proteins in the serum

Principle:

Proteins in the serum reacts with Biuret reagent to form a purple coloured complex which is measured photometrically .

Referrence interval:

Adult 6.4 -8.3 gms/dL

All individuals included in this study had their protein values within the reference range.

Estimation of serum Albumin:(49)

METHOD:

BROMO CRESOL GREEN METHOD (ENDPOINT)

Aim:

To determine the amount of albumin present in the serum

Principle:

At pH 3.68. albumin acts as a cation and binds to the anionic dye, Bromo Cresol Green (BCG) forming a green coloured complex which can be read photometrically.

Referrence interval:

Adults - 3.2 – 5.2gms/dL

HEMOGLOBIN A1C ESTIMATION : (50)

METHOD: TURBIDIMETRIC IMMUNO INHIBITION ASSAY

Instrument: Cobas C 311 Analyser.

FIG. 11 : COBAS C 311 ANALYZER

Aim:

To determine the HbA1C in anticoagulant added whole blood.

Principle:

The hemolysed sample containing HbA1C reacts with antibody to N terminal portion of the beta chain of hemoglobin to form soluble antigen antibody complexes.

A poly hapten added reacts with excess of antibody forming insoluble Ag - Ab compexes which can be measured photometrically.(Turbidimetry)

Specimen collection and processing:

Whole blood from vein or capillary collected in Na or K EDTA vacutainers.

Samples are analysed on the same day of collection and preserved in 2 - 8 ° C until the time of analysis.

Preparing hemolysate:

The hemolysing reagent and Sample were taken in the ratio of 1 : 100, mixed in shaker or vortex mixer at room temperature for 1-2 minutes.

Hemolysate was stable for 6 months at -20°C.

Reagent composition:

R1- Antibody reagent:

MES buffer 0.025mol/L

TRIS Buffer 0.015 mol/L, pH 6.2

HbA1C Ab (bovine serum)≥ 0.5 mg/mL

Stabilizers

Preservatives

R2 - Polyhapten reagent

MES Buffer 0.025 mol/L

TRIS Buffer0.015mol/L pH 6.2

HbA1C polyhapten ≥8µg/mL

Stabilizers and Preservatives

R3 Hemolyzing reagent:

Contains TTAB as detergent

Assay parameters: HbA1C- W2

Assay type : 2‑Point End

Reaction time : 10 minutes

Assay points : 23‑57

Wavelength (sub/main): 660/340 nm

Reaction direction : Increase

Unit : g/dL

Reagent pipetting Diluent (H2O)

R1 120 µL –

R2 24 µL –

ASSAY PARAMETERS Hb-W2

Assay type 2‑Point End

Reaction time – 10 minutes

Assay points - 23

Wavelength -(sub/main) 660/376 nm

Reaction direction- Increase

Unit g/dL

Reagent pipetting	Diluent (H2O)
R1 120 µL	–
R2 24 µL	–

Calculation:

Estimated average glucose:

[mmol/L] = 0.146 × HbA1c (mmol/mol) + 0.834 or

Estimated average glucose:

[mg/dL] = 28.7 × HbA1c (%) - 46.7

HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

(mmol/mol HbA1c acc. to IFCC)(17)

HbA1c (mmol/mol) = (HbA1c/Hb)x 1000

Reference Interval:

HbA1C : 4.3‑18.9 %

Hemoglobin: 4‑35 g/dL

STANDARDISATION:

6 Point calibration was done with calibrator for HbA1C

The standard graph is used for calculating the sample values.

Quality control:

Quality control is performed using the control provided with the kit for high and low values.

Estimation of Plasma glucose: (Fasting)

INSTRUMENT:

Merck Microlab 300

Method: Trinder's method (51)

Principle:

1. Glucose present in the sample is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide.

2. Hydrogen peroxide reacts with phenolic compound in the presence of amino- anti pyrine to form a coloured complex .

Reference interval:

Adult: < 100mg/dL

Pregnancy - <95mg/dL

ESTIMATION OF SERUM FRUCTOSAMINE:

INSTRUMENT:

FULLY AUTOMATED ANALYZER

Fig.12 : AU 480 BECKMAN COULTER

METHOD:

Modified nitroblue tetrazolium method (by Baker et al) (52)

Aim:

To determine the concentration of fructosamine present in the serum of the given sample.

Principle:

Fructosamine reduces Nitroblue tetrazolium in alkaline conditions to form a coloured formazan compound which is measured photometrically at 530 nm. The intensity of the colour developed is proportional to the concentration of fructosamine in the serum.

Specimen collection and processing:

Serum separated from clotted whole blood was analysed.

Samples were stable at -20°C for 2months.

Once thawed, the sample was not refreezed.

Reagent composition:

Nitroblue tetrazolium -- 0.48 mM

Triton X 100 -- 2.2 gm%

Sodium carbonate - 100 mM pH 10.3

Reagent preparation:

1. Buffer stock:

a) Sodium Carbonate 1M (100ml)

106 gms of dried sodium carbonate is weighed and transferred into a clean glass beaker

80 ml of deionized water is added and mixed well.

The contents are transferred into a clean dry 100ml volumetric flask and made upto 100ml.

Stored in refrigerator at 2-8°C.

b) Sodium hydrogen carbonate 1 M:(100ml)

84 gms of dried sodium hydrogen carbonate is weighed and made upto 100 ml in a volumetric flask , mixed well.

Transferred and stored at 2-8°C.

Working buffer:

1 in 10 dilution of buffer stock was prepared from 1M solutions of Sodium carbonate and sodium bicarbonate to obtain 0.1M solutions.

Standardization of pH Meter:

After washing the probe with deionized water, it was placed in deionized water for 30 minutes.

Standard solutions of pH 7.0 and 10.0 were used to standardize the pH Meter. Reagent preparation: (100 ml)

25 ml of sodium carbonate 0.1 M solution was taken in a clean glass beaker and 65 ml of sodium hydrogen carbonate 0.1 M solution was added.

The pH is measured and adjusted to 10.3 by titrating with sodium hydrogen carbonate 0.1M and made upto 95 ml in a volumetric flask.

The NBT powder was weighed (41 mgs),taken in a beaker, dissolved with 2-3 ml of deionized water and 2.2 gms of the detergent. It was mixed well and transferred into the volumetric flask containing buffer.

The final volume of 100 ml was made and final pH verified.

The reagent was ready to use.

Storage and precautions:

The reagent was stored in a dark brown bottle .

Temperature : 2-8°C

Stable for 5 – 7 days.

Direct bright light should be avoided as it deteriorates the NBT activity.

Procedure:

Both reagent and serum to be analyzed should be brought to room temperature.

Prewarming of the reagent volume to be used is done while using spectrophotometer or semi- auto analyzer.

Dilution (1:5) is made for analyzing in spectrophotometer and calculation is done accordingly.

Assay parameters:

Mode	:	Kinetic
Wavelength	:	530nm
Reagent volume	:	500µL
Sample volume	:	25µL
Reaction direction	:	increasing
Blank	:	reagent blank
Linearity	:	upto 800mM

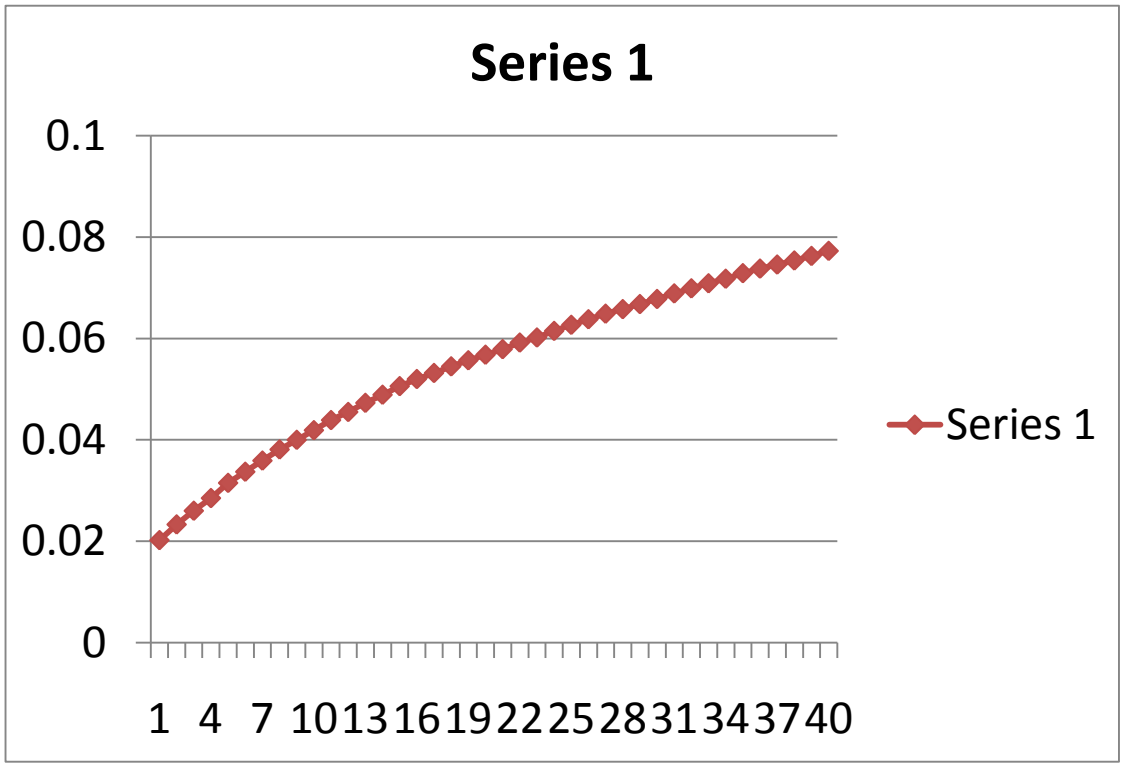
Calculation:

$$\text{Fructosamine in serum in µmol/L} = \frac{\Delta \text{ Absorbance of the test}}{\Delta \text{ Absorbance of the std.}} \times \text{conc.of the std.}$$

Standardisation of the procedure:

The procedure is standardized using commercial fructosamine standard .

FIG – 13 : SERUM FRUCTOSAMINE KINETICS:



TIME IN MINUTES

Quality control:

Standards of known concentration prepared from the calibrator is used for verifying the test results.

ESTIMATION OF NAIL FRUCTOSAMINE:

METHOD:

NITROBLUE TETRAZOLIUM METHOD (BAKER et al.)(44)

INSTRUMENT:

UV 1800 SPECTRPHOTOMETER(Schimadtzu)

Fig.14 : SPECTROPHOTOMETER

Aim:

To determine the fructosamine levels in the human finger nail clippings.

Principle:

The fructosamine formed in the nail protein keratin and the ventral nail plate throughout its growth period across the nail bed was determined from the nail clippings taken from the free edge of the finger nail by its ability to reduce the nitroblue tetrazolium to a coloured formazan compound .This was measured photometrically at 530 nm. The intensity of the colour formed was directly proportional to the fructosamine present in the given nail sample. The assay was based on the property of the nail being susceptible to less hydrophilic substances to diffuse through the nail and the diffusion rate being uniform due to thickness of the nail plate being uniform with minimal inter individual variations.

Specimen collection and processing:

FIG.15 : NAIL – SPECIMEN PROCESSING

The selected patient population was instructed to clean the nails by washing with soap and water as usual at their homes.

Nail clippings were taken using a nail clipper .

For uniformity , the nails from four fingers except thumb are taken for the assay.

The finger nails known to be coated with substances eg. Nail polish, mehenti were avoided.

The patients included in the study were asked to avoid such decorative works and biting the nails during their follow-up period of 10 – 12 weeks.

The nail clippings and venous blood for HbA1c were taken simultaneously.

Processing and storage:

The clippings were preserved in 0.1 ml microfuge tubes.

It can also be collected in plain white paper (without print ink or anycolour) and preserved in clean plastic boxes.

They can be preserved at room temperature for about three months.

Moisture and contaminants were avoided during storage.

Before testing the nails were cut into tiny bits of 2-3 mm approximately.

The nail bits were weighed in a balance which was standardized using 100mg and 5mg standard weights.

The weighed nail substance was transferred into clean glass test tubes after labelling.

One ml of prewarmed NBT reagent (room temperature) was added to each of the test tubes.

They were arranged in a rack and incubated at 37°C for one hour.(Timer set)

After 30 minutes of incubation, the test tubes were shaken and mixed.

The tubes were removed ensuring completion of one hour incubation for all the samples.

Readings were taken in a spectrophotometer with reagent in the reference cell.

Preparing the spectrophotometer:

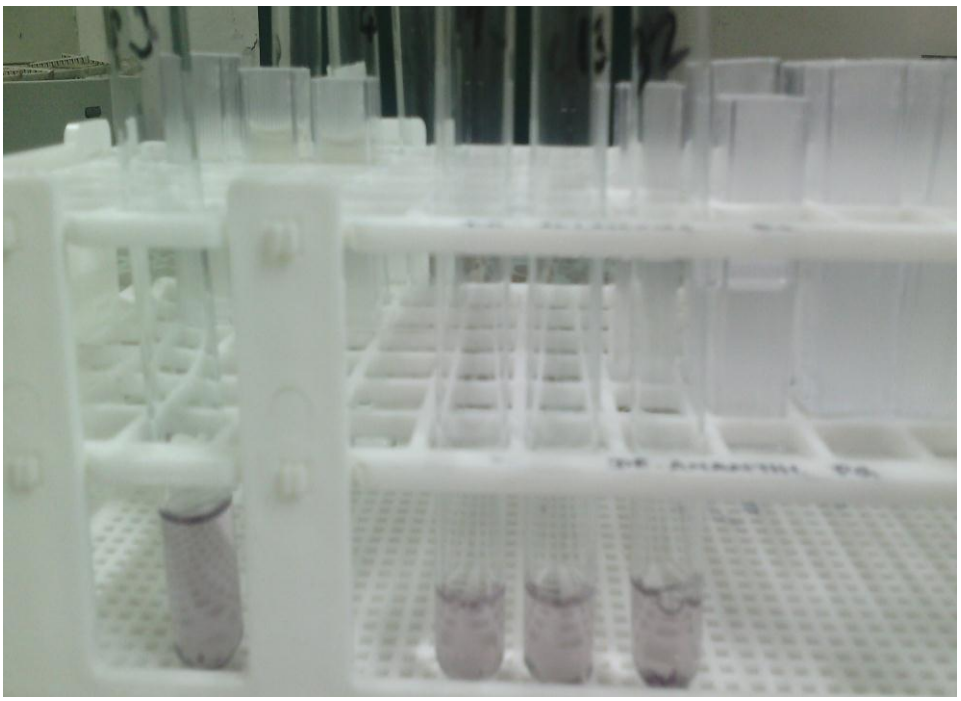
While after completion of 45 minutes of the reaction time, the spectrophotometer was switched on and self check time allowed.

Spectrometer was programmed as for photometric settings.

Assay parameters:

Mode	:	End point
Wavelength	:	530 nm
Reaction time	:	1 hr
Reaction temperature	:	37°C
Reagent volume	:	1 ml
Sample by weight	:	10 mg
Unit	:	µmol/L/mg
Cuvette volume	:	1.5 ml

FIG.16 : THE END POINT IN NAIL FRUCTOSAMINE ASSAY



Calibration:

Fructosamine standard in human albumin preparation is used to standardize the assay.

The concentration is 404µmol/L

Preparation of standards:

The standard used should be within or close to the reference interval.(53)

Hence from the calibrator 8µmol standard is prepared and used as standard.

The concentration is 404µmol/L

Preparation of standards:

The standard used should be within or close to the reference interval.

Hence from the calibrator 8µmol standard was prepared and used as standard.

The standard solution was weighed and 10mg eqiuvalent was taken for the assay for standardization.

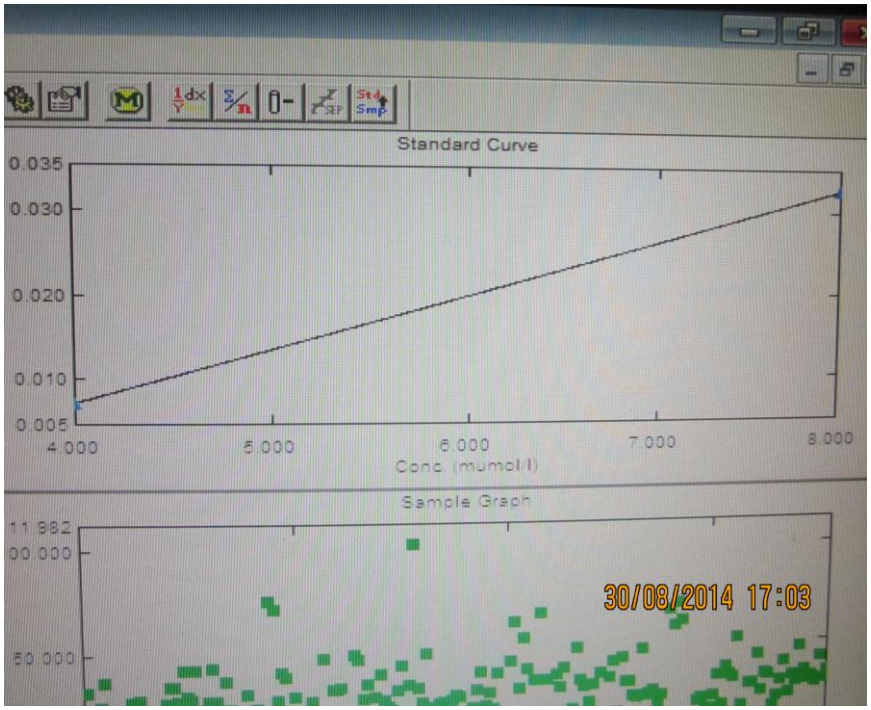
Dilutions were prepared using normal saline.

The standard solutions were brought to room temperature prior to use.

The required volumes were prepared freshly prior to use to avoid repeated freezing and thawing.

STD GRAPH:

FIG.17 - STANDARD GRAPH NAIL FRUCTOSAMINE ASSAY



Calculation:

The quantity of fructosamine in nail is calculated using the following formula

$$\text{Fructosamine present in the nail /gm of the substance} = \frac{\text{Absorbance of test}}{\text{Absorbance of the standard}} \times \text{conc. of the standard}$$

Concentration of standard = 8µmol/L

Unit conversion:

The result obtained per 1000µL for 10mg is in µmol/ Kg equivalents.

The conversion is done for g equivalents by multiplying it by1000.

Referrence interval:l(54)

Male: 0.58 – 3.8µmol/gm equivalent of nail

Female: 0.55 – 3.32µmol/gm equivalent of nail

RESULTS AND STATISTICAL ANALYSIS

The present study is a 6 months longitudinal study of diabetic patients in Stanley Medical College and Hospital, Chennai from the month of April 2014-September2014.The long term glycemic control of diabetic patients were analyzed using a newer parameter-glycated nail protein (fructosamine) in finger nails and the results were compared with the controls.

ANALYSIS OF CASES

All known diabetic patients who were taken in to the study were analyzed completely beginning with history taking which involved age,sex, duration of the illness, family history of diabetes. General examinations like pulse rate, blood pressure, height, weight were taken. Patient's risk factors were analyzed in terms of lipid profile and BMI and associated co morbidities were also analyzed..

Fasting plasma glucose, HbA1C levels, serum fructosamine levels and nail fructosamine levels were taken for analysis to assess their glycemic status during the period of study. Plasma glucose and serum fructosamine were taken as mean values. HbA1C and Nail fructosamine were performed twice (initial and final) for individual comparison.(55).Both these parameters were also compared and analyzed.

TABLE : 9 - DISTRIBUTION OF AGE GROUP

AGE GROUP	NUMBER OF PATIENTS
0-10	0
11-20	0
21-30	1
31-40	5
41-50	12
51-60	8
61-70	9
71—80	0
81-90	0
91-100	0

In the present study 35 diabetic patients were studied . Most of the patients belonged to the age group of 41-50. The **mean age of the study group was 51.1years.**

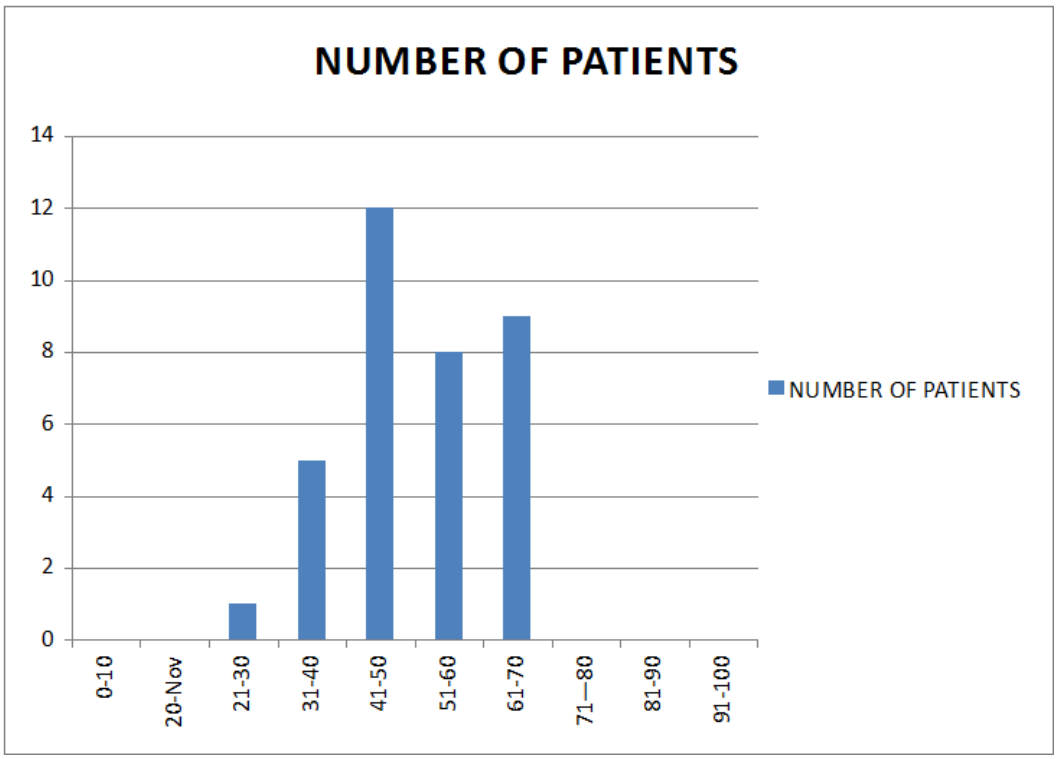


FIG.18 - AGE DISTRIBUTION

TABLE - 10 : SEX DISTRIBUTION

PATIENT'S SEX	NUMBER
MALE	8
FEMALE	27

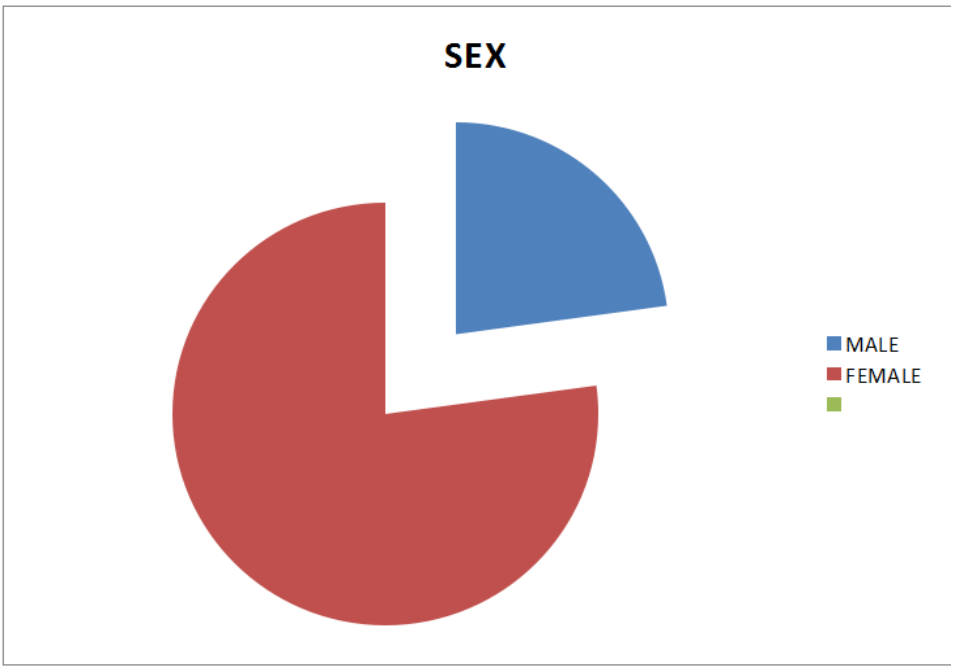


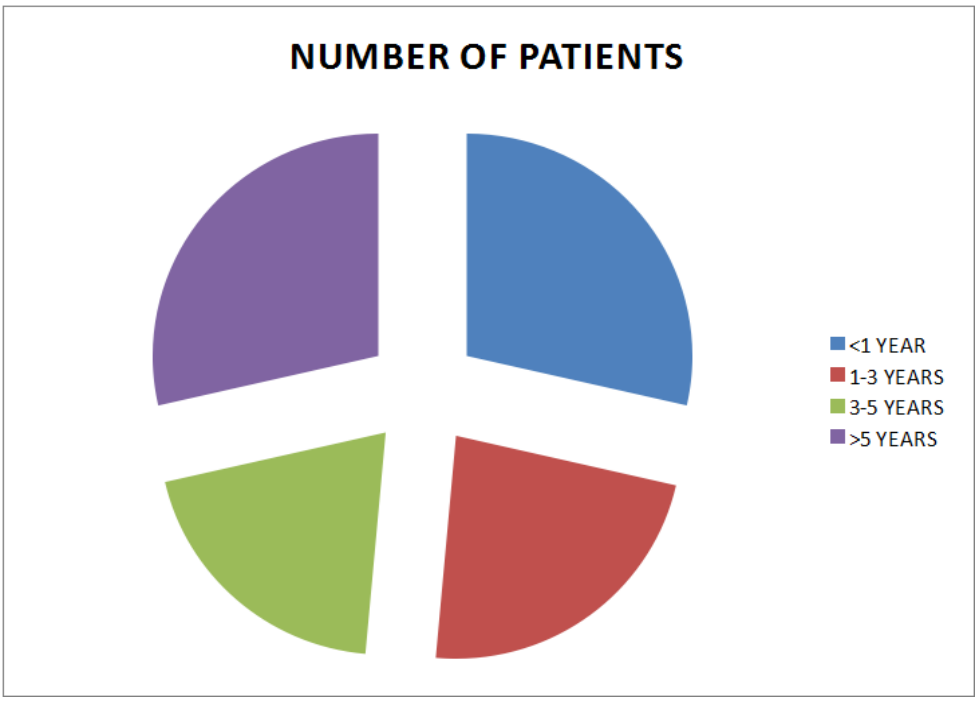
FIG.19 - SEX RATIO - CASES

In the above study females constituted 77% of the study population among patients.

TABLE – 11 : DURATION OF THE DISEASE

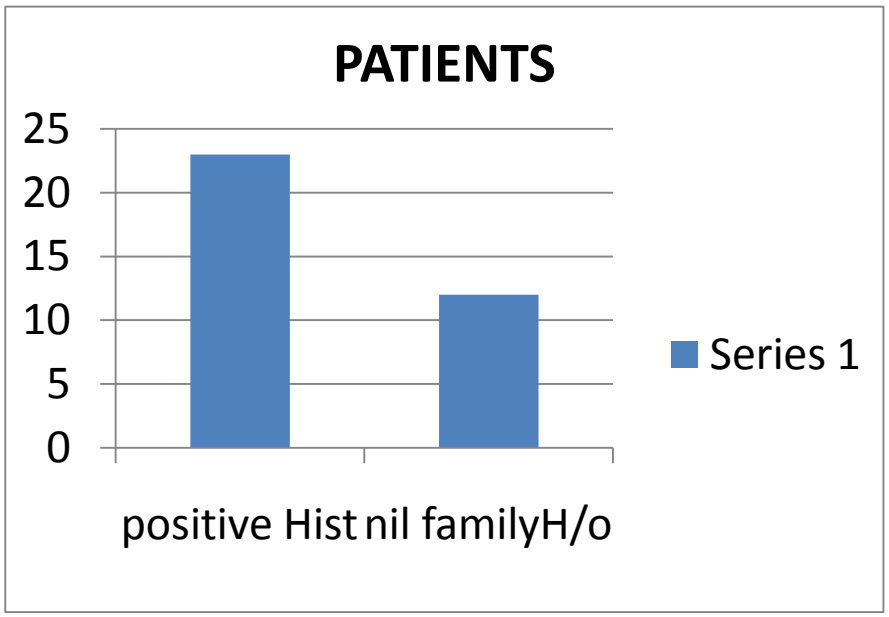
DURATION	NUMBER OF PATIENTS	PERCENTAGE
<1 YEAR	10	28.6
1-3 YEARS	8	22.9
3-5 YEARS	7	20
>5 YEARS	10	28.5

FIG.20 : DURATION OF DIABETES



In the present study, duration of the diabetes in the group belonged maximum to less than 1 year(28.6%) and >5years (28.5%).

FIG.21 : FAMILY HISTORY



In the present study family history was positive in 65.7% of the patients indicating the influence of genetic factors .The individuals who served as controls have equally significant positive family history and a chance of developing the disease.

DRUG INTAKE:

TABLE – 12 : DRUG INTAKE

DRUGS	PATIENTS
Insulin and Oral hypoglycemic agents	3
NIL	0
Oral hypoglycemic agents	31
Oral hypoglycemic agents	1

In the present study most of the patients were in oral hypoglycemic drugs mainly(88.57%). Others were given oral drugs in a combination with insulin.

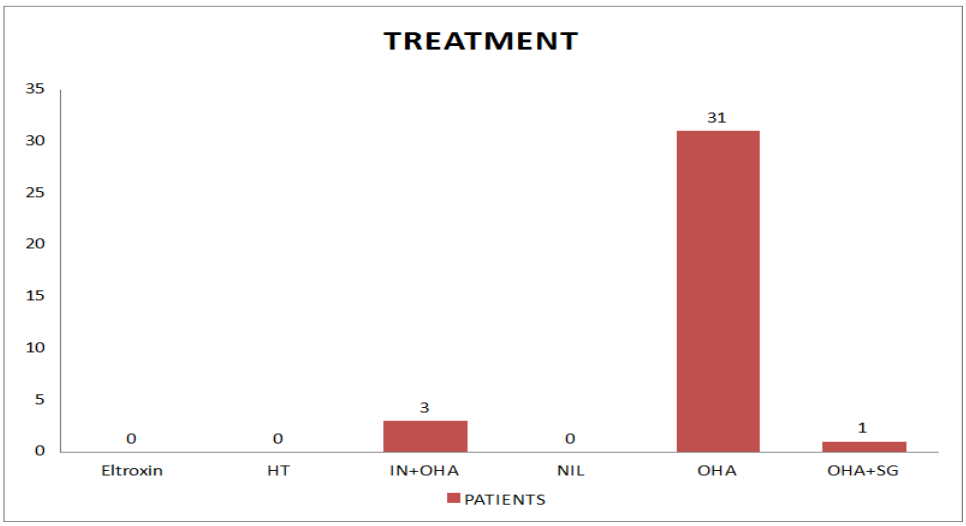


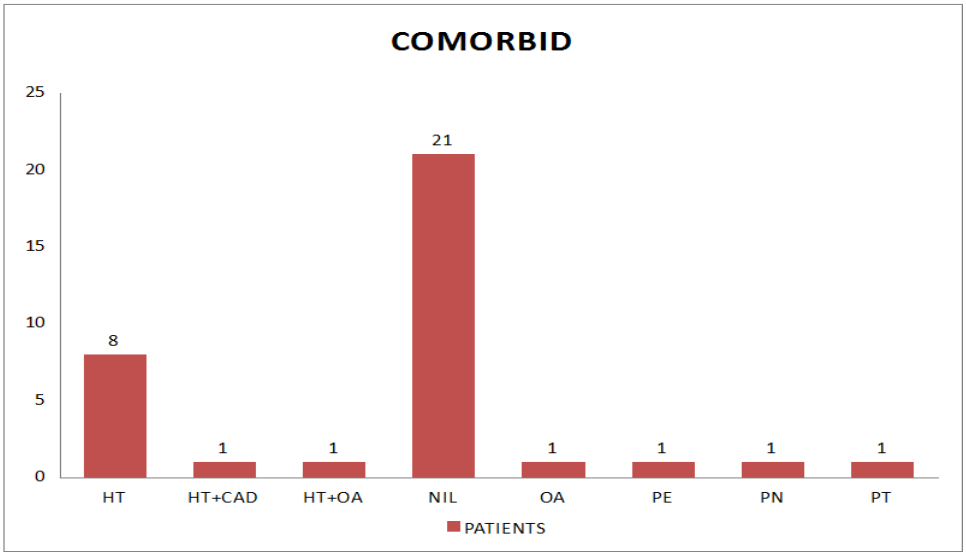
FIG. 22 : DRUG THERAPY - PATIENTS

ANALYSIS OF COMORBID CONDITIONS:

TABLE - 13

COMORBID CONDITION	NUMBER OF PATIENTS
Vascular diseases	1
Hypothyroidism	3
Osteo-arthritis	1
Hypertension	8
Peripheral neuropathy	1
Pedal edema	1
Nil	20

FIG.23 - VARIOUS ASSOCIATED COMORBIDITIES - CASES



In the present study overall comorbidity was seen in 42.85% of cases. Of them hypertension was found to be the most common associated comorbidity(22.8%) followed by hypothyroidism.

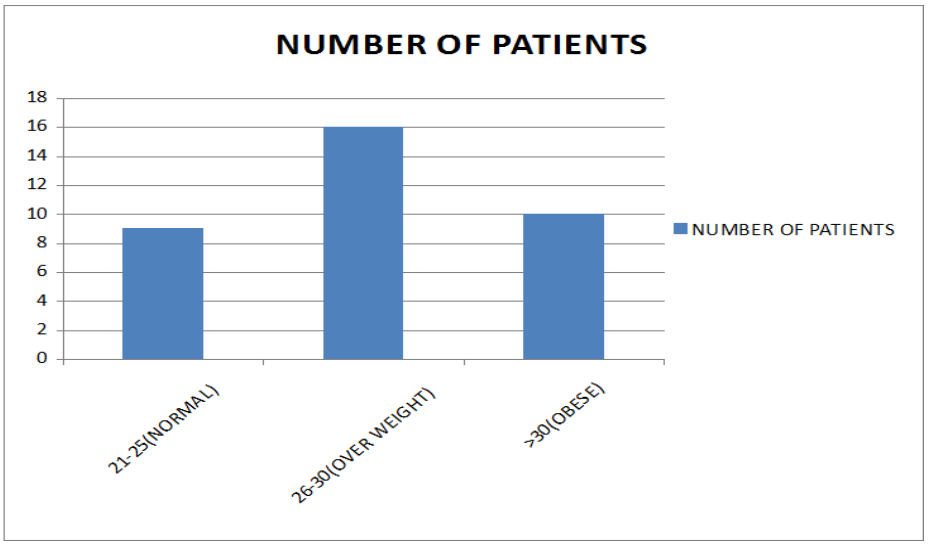
ANALYSIS OF BMI :

In the present study analysis of BMI was done in every patient and risk factor assessed. Most of the patients (45.71%) were found to be in the category of over weight

TABLE – 14 : BMI

BMI	NUMBER OF PATIENTS
21-25(NORMAL)	9
26-30(OVER WEIGHT)	16
>30(OBESE)	10

FIG.24 : BODY MASS INDEX IN PATIENTS



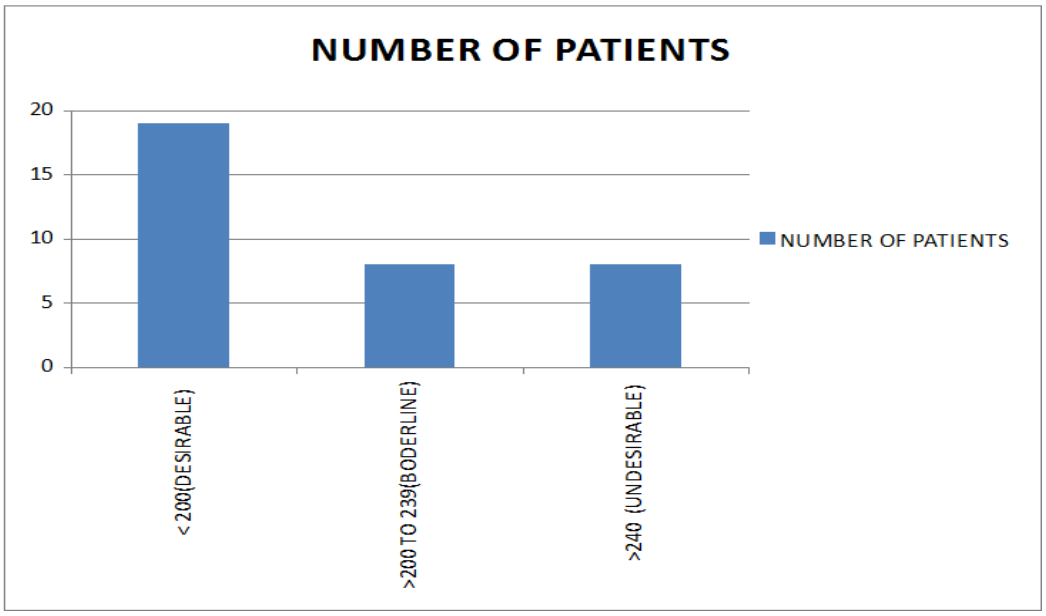
ANALYSIS OF LIPID PROFILE :

In the present study an analysis of lipid profile was made

CHOLESTEROL LEVEL	NUMBER OF PATIENTS
< 200(DESIRABLE)	19
>200 TO 239(BODERLINE)	8
>240 (UNDESIRABLE)	8

TABLE .15 : CHOLESTEROL LEVELS

FIG.25 : CHOLESTEROL LEVELS



When the total cholesterol levels were analyzed, half of the patients (54%) were found to have a normal range. 22.85 % had their cholesterol levels in the high risk range.

TGL ANALYSIS :

TGL	NUMBER OF PATIENTS	PERCENTAGE
50 TO 160 (NRL)	22	62.85
>160(ABNRL)	13	37.15

TABLE : 16 - TGL -CASES

62.85% of the patients were found to have TGL in the normal range.

LDL ANALYSIS:

TABLE - 17 : LDL LEVELS

LDL	NUMBER OF PATIENTS	PERCENTAGE
< 130 (NRL)	21	60
130 TO 160(LOW RISK)	6	17
>160 (HIGH RISK)	8	23

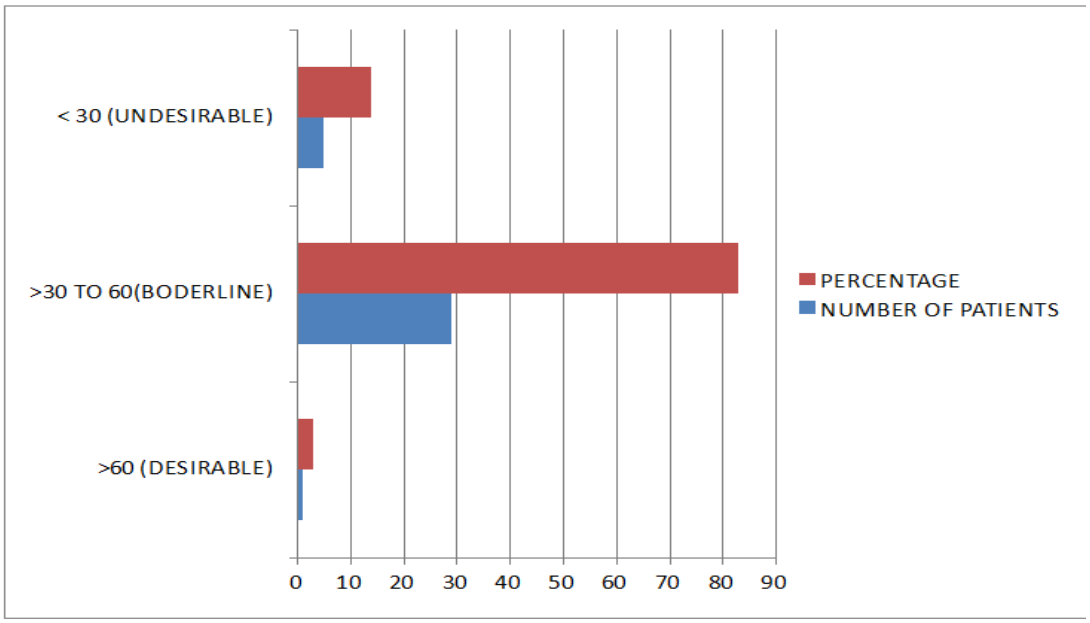
LDL levels were found to be abnormal in 40% of the patients.

HDL ANALYSIS :

TABLE – 18 : HDL LEVELS

HDL	NUMBER OF PATIENTS	PERCENTAGE
>60 (DESIRABLE)	1	3
>30 - 60(BODERLINE)	29	83
< 30 (UNDESIRABLE)	5	14

FIG .26 - HDL CHOLESTEROL LEVELS



However HDL were found to be in the borderline for most of the patients(83%).

HDL/CHOLESTEROL RATIO ANALYSIS :

TABLE : 19 - HDL/CHOLESTEROL RATIO

HDL/CHOLESTEROL	NUMBER OF PATIENTS	PERCENTAGE
NO RISK > 37	2	5.7
LOW RISK - 19 TO 36	17	48.6
HIGH RISK – 7 TO 18	16	45.7

Most of the cases were found to be in the low risk (48.6%) & high risk (45.7%) category in relation to HDL/CHOLESTEROL ratio.

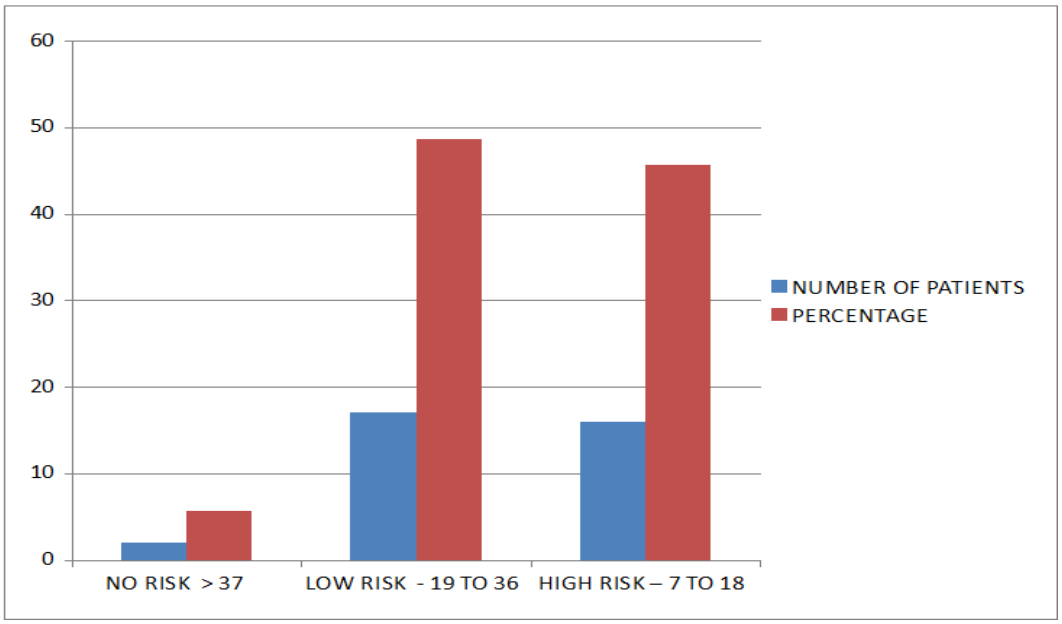


Fig. 27 - HDL/TOTAL CHOLESTEROL- Risk attribution

ANALYSIS OF CONTROLS

All the controls were analyzed for all the parameters as that of cases.

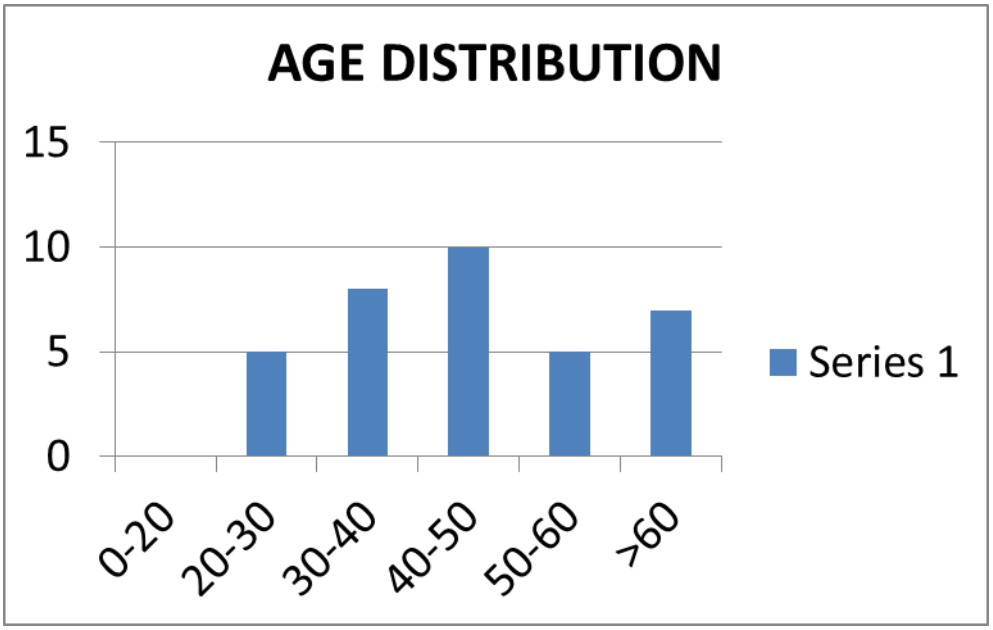
DISTRIBUTION OF AGE

In the control group most of them belonged to the age group of 30-50 years.

TABLE – 20 : AGE DISTRIBUTION

Age in years	No. of patients
0-20	0
20-30	5
30-40	8
40-50	10
50-60	5
>60	7

FIG.28 : AGE DISTRIBUTION



SEX DISTRIBUTION:

TABLE : 21 - SEX DISTRIBUTION

SEX	NUMBER OF PATIENTS
MALE	9
FEMALE	26

In the present study females constituted 74% of the total control population.

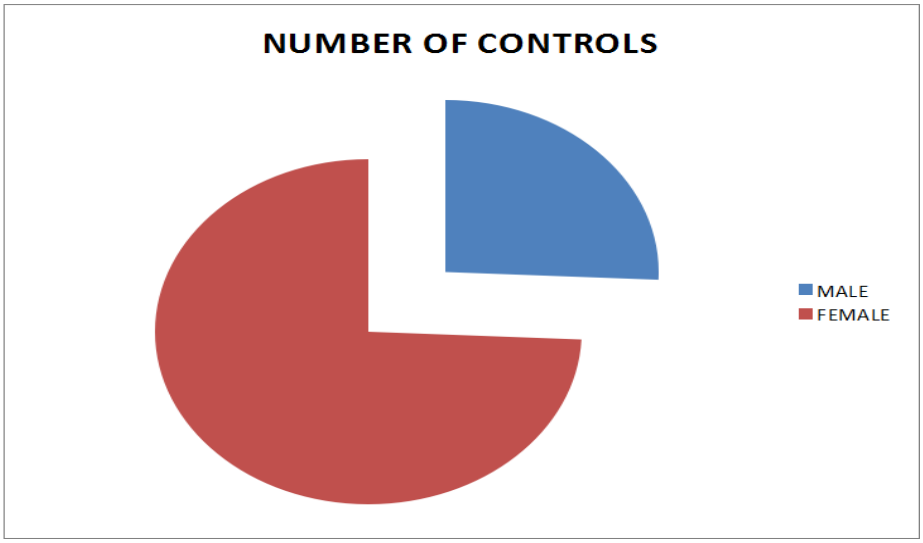


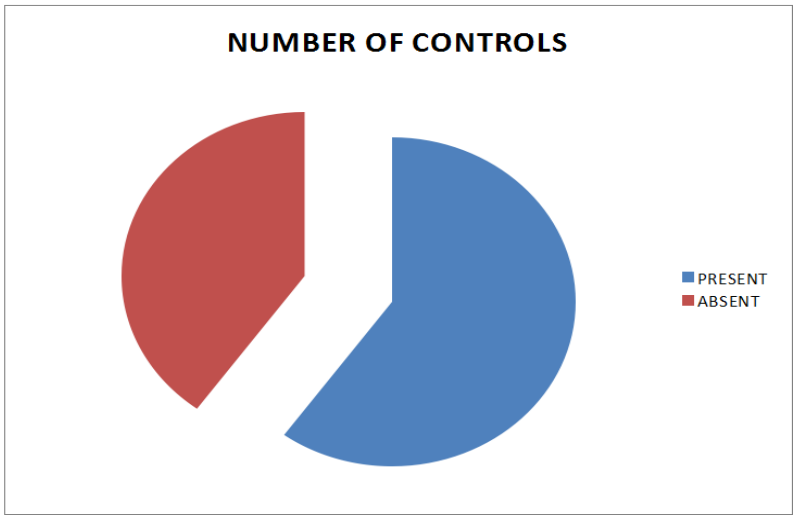
FIG. 29 - SEX RATIO – CONTROL GROUP

ANALYSIS OF FAMILY HISTORY:

TABLE - 22 : POSITIVE FAMILY HISTORY

FAMILY HISTORY	NUMBER OF PATIENTS
PRESENT	21
ABSENT	14

FIG - 30 : FAMILY HISTORY



In the present study family history was positive in 60% of controls.

The other parameters like pulse rate, blood pressure, BMI, lipid profile were normal in case of controls. The associated co morbidities was only hypothyroidism in 3 cases of controls. The glycemic indices like serum fructosamine, nailfructosamine, HbA1C were also normal in these individuals. A minimal overlap is also noticed in nail and serum fructosamine.

COMPARISON OF CASES AND CONTROLS:

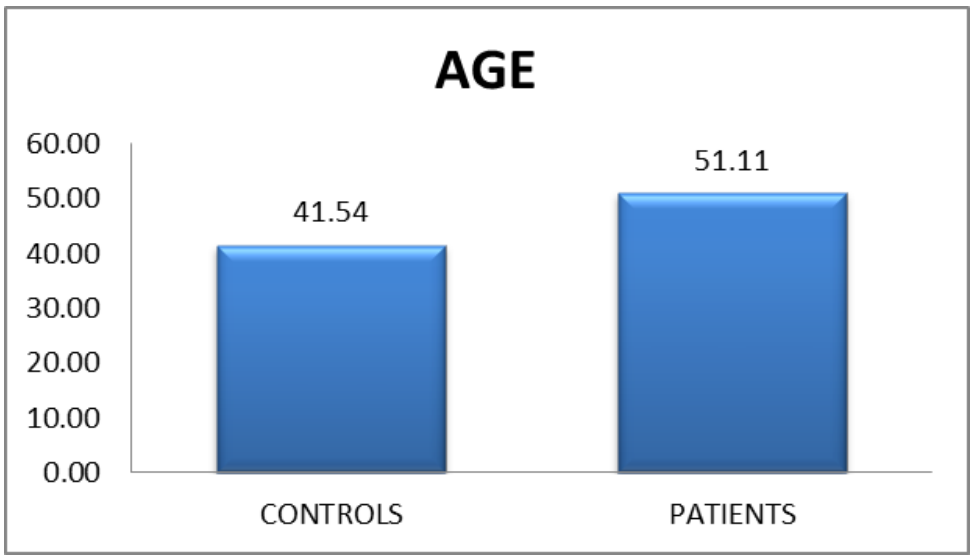
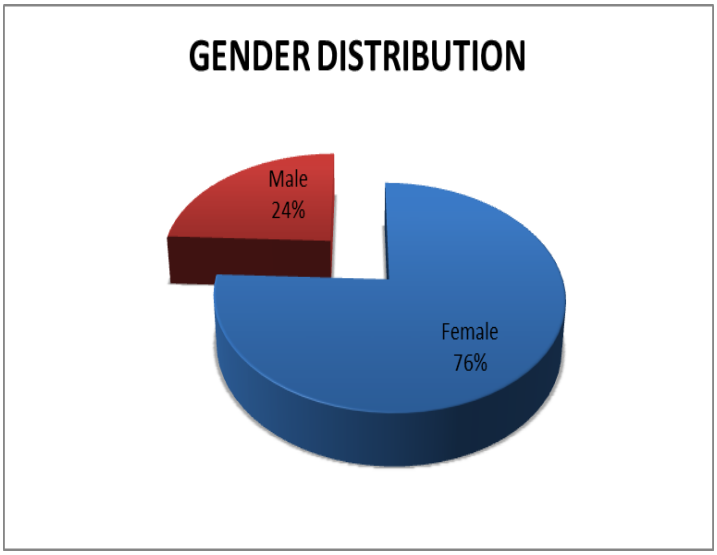


FIG.31. AGE IN YEARS –COMPARISON OF MEANS

The mean age of the case and control group were 51.11 and 41.54 respectively. The range is between 22 and 72 for cases . The range for controls is 28 –72. Females formed the predominance of study group in both cases and controls.

FIG.32 : GENDER DISTRIBUTION

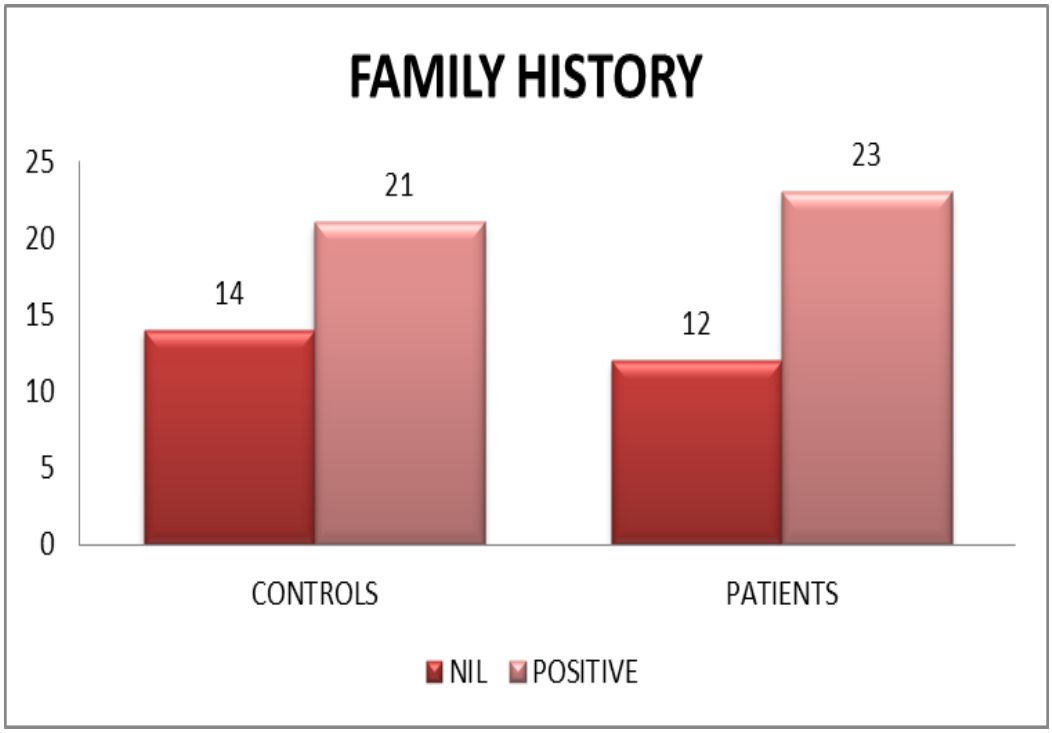


Both group had proportionately similar family history of ≥ 60% with no significant difference.

TABLE - 23 : FAMILY HISTORY

FAMILY HISTORY	CONTROLS	PATIENTS	Total
NIL	14 40.0%	12 34.3%	26 37.1%
POSITIVE	21 60.0%	23 65.7%	44 62.9%
P value	0.621		
SIGNIFICANCE	NIL		

FIG – 33 : FAMILY HISTORY



There is no significant difference in positive family history between patients and controls.

COMRBID CONDITIONS

The most common co-morbid condition in the patients was hypertension while in control group there was no significant associated co- morbidity except for hypothyroidism.

TABLE – 24 : COMPARISON OF CO-MORBID CONDITIONS

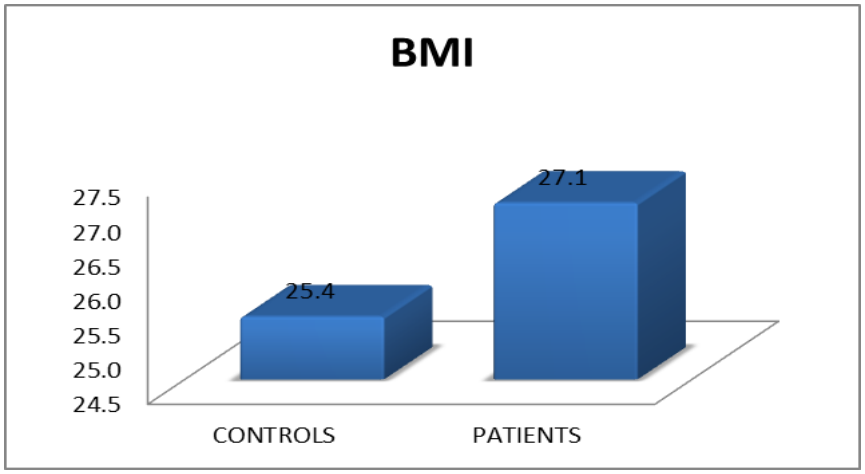
CO-MORBID CONDITION	CONTROLS	PATIENTS	Total
HYPERTENSION	3 8.6%	8 22.9%	11 15.7%
HYPERTENSION & CORONARY DISEASE	0 0.0%	1 2.9%	1 1.4%
HYPERTENSION & ARTHRITIS	0 0.0%	1 2.9%	1 1.4%
NO CO-MORBID	31 88.6%	21 60.0%	52 74.3%
ARTHRITIS	1 2.9%	1 2.9%	2 2.9%
PEDAL EDEMA	0 0.0%	1 2.9%	1 1.4%
PERIPHERAL NEUROPATHY	0 0.0%	1 2.9%	1 1.4%
PULMONARY TUBERCULOSIS	0 0.0%	1 2.9%	1 1.4%

The overall co-morbidity is 40% in patients and 11.4% in controls.

BODY MASS INDEX:

The average BMI of the case group was 27.1 (overweight) while that of controls was 25.4 (normal range), however the difference was weakly significant.(p value 0.09)

FIG.34 : BMICOMPARISON



COMPARISION OF LIPID PROFILE :

The lipid profile was done for each group and the results were analysed. The mean value for cholesterol in cases and controls were 196 and 185 respectively. There was no significant difference between the two groups (p value 0.337).

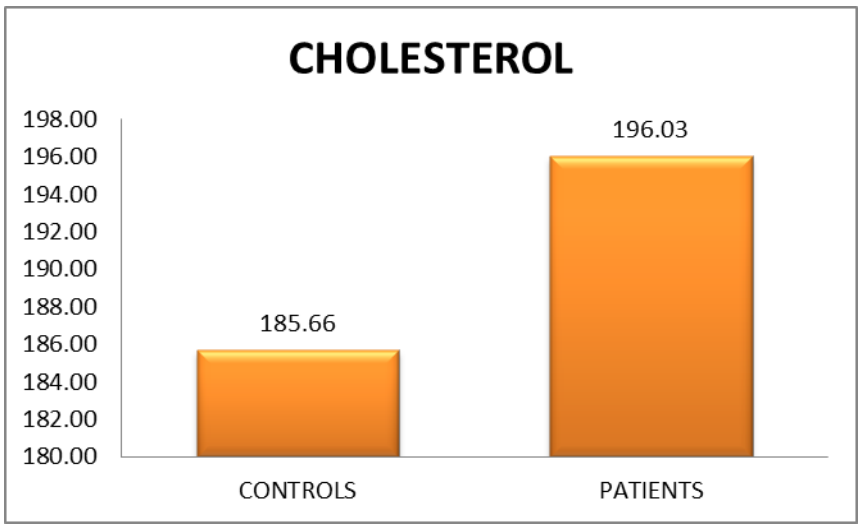
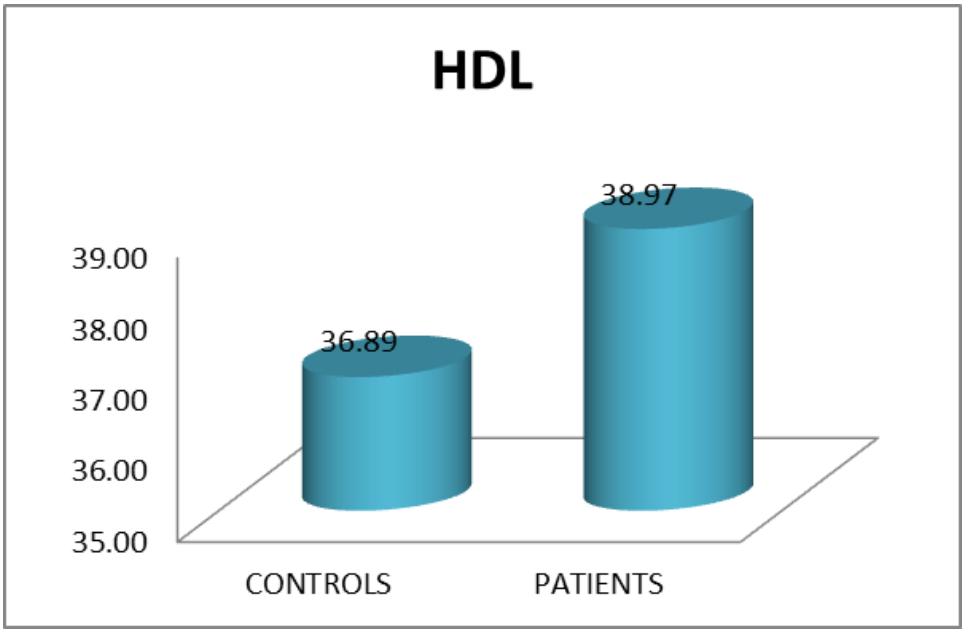


FIG. 35- COMPARISON – CASES AND CONTROLS – TOTAL CHOLESTEROL

HDL CHOLESTEROL :

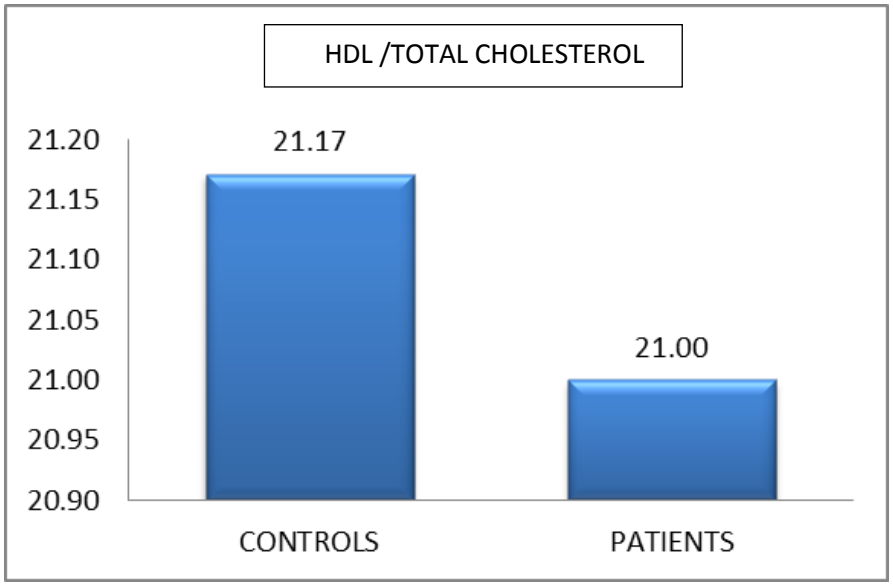
The HDL level of both groups were compared. The mean HDL levels of each group was 38.97 and 36.89. Though there was no significant difference between both groups, their levels were in the borderline risk range.(30-60)

Fig. 36 : HDL CHOLESTEROL COMPARISON CASE AND CONTROLS



HDL/CHOLESTEROL RATIO : The above ratio was 21 and 21.17 in cases and controls respectively. In both groups the mean value was found to be in the low risk group. Also **no significant** difference was noted in HDL values between cases and controls.

Fig .37 : HDL / TOTAL CHOLESTEROL

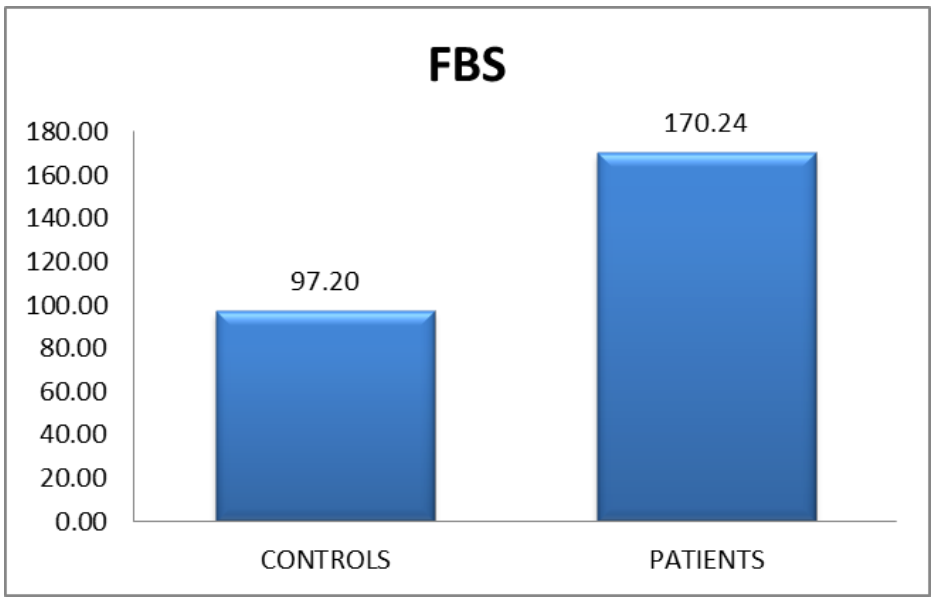


THE GLYCEMIC INDICES:

COMPARING THE MEANS:

FASTING BLOOD SUGAR: FBS

FIG .38 : COMPARISON OF FBS MEAN



The difference is significant.

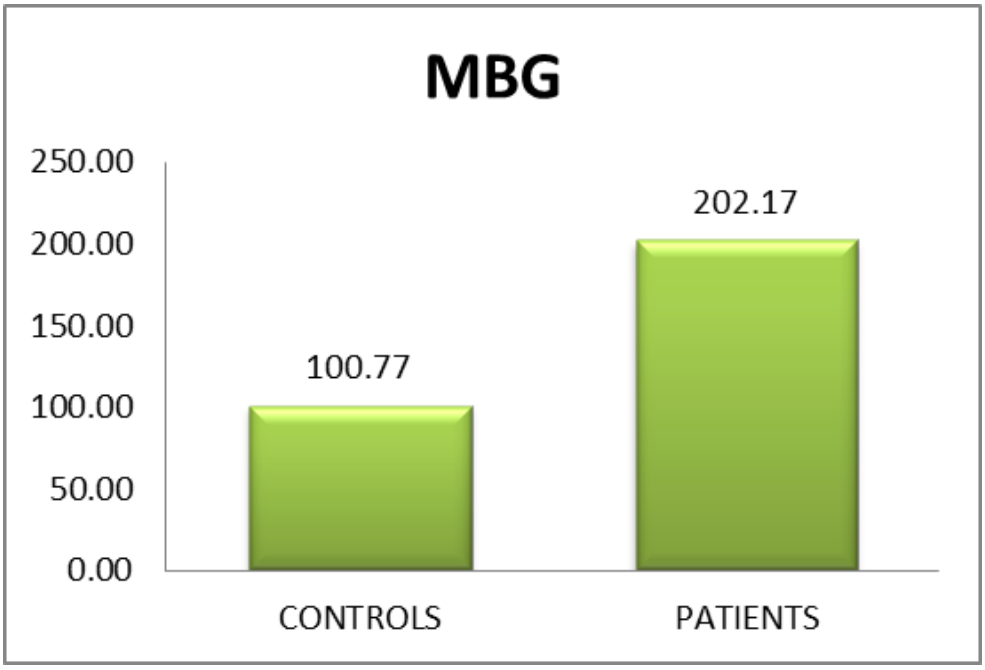


FIG. 39 : AVERAGE DERIVED GLUCOSE - MEAN

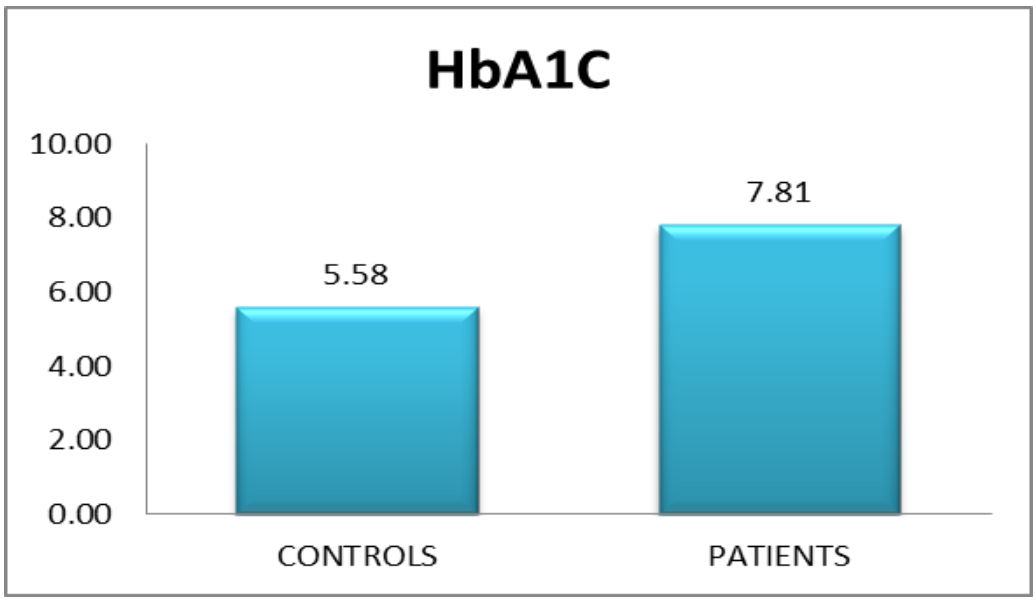


FIG.40 : HBA1C – MEAN

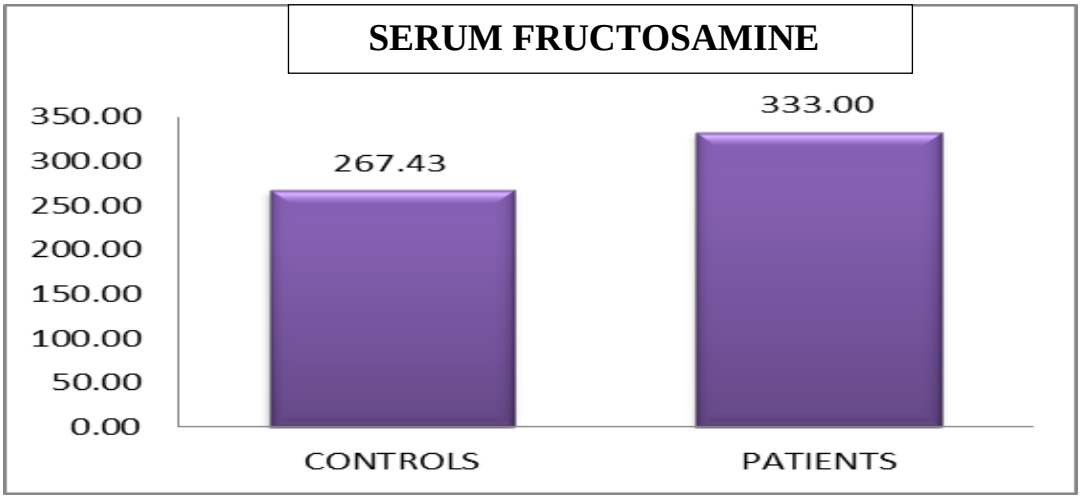


Fig.41 : SERUM FRUCTOSAMINE MEAN

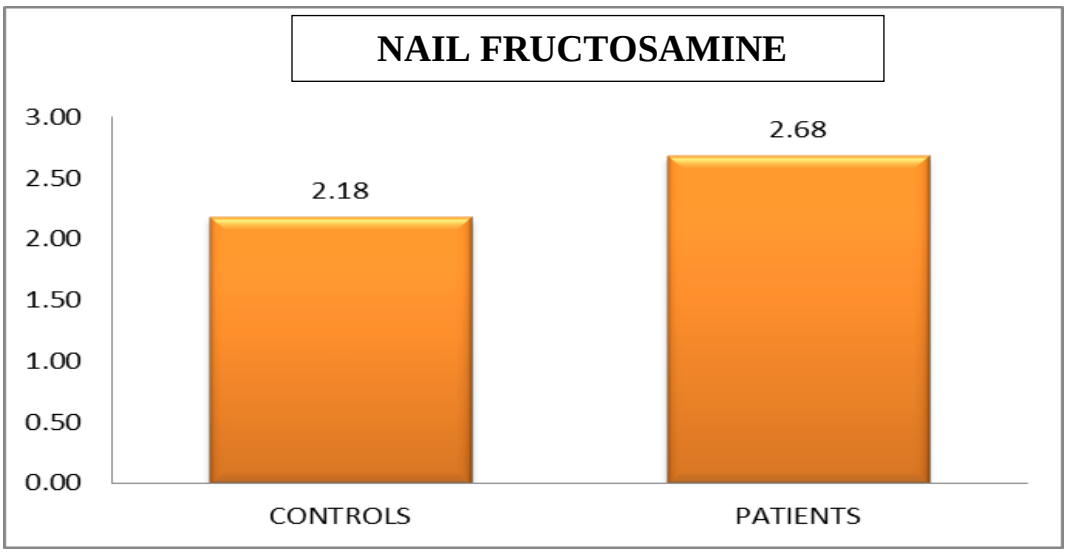


Fig.42 : NAIL FRUCTOSAMINE MEAN

TABLE - 25 : COMPARISON OF MEANS – GLYCEMIC INDICES

S.NO	VARIABLE	MEANCONTROLS PATIENTS	Mean PATIENTS	T value	P value	Significance
1	FBS MEAN	97.2	170.24	-8.967	0.0001	Strong significance
2.	MEAN BLOOD GLUCOSE	100.77	202.17	-8.388	0.0001	Strong significance
3.	HbA1C	5.583	7.806	-8.632	0.0001	Strong significance
4.	SERUM FRUCTOSAMINE	267.43	333	-5.562	0.0001	Strong significance
5.	NAILFRUCTOSAMINE	2.177	2.679	-2.706	0.009	significant

ANALYSIS OF GLYCEMIC STATUS OF CASES AND CONTROLS:

The set of initial and final values of patients were compared .

Both show a decrease in the mean indicating a glycemic control has been achieved.

Also the results well correlate with Fasting blood glucose and serum fructosamine levels.

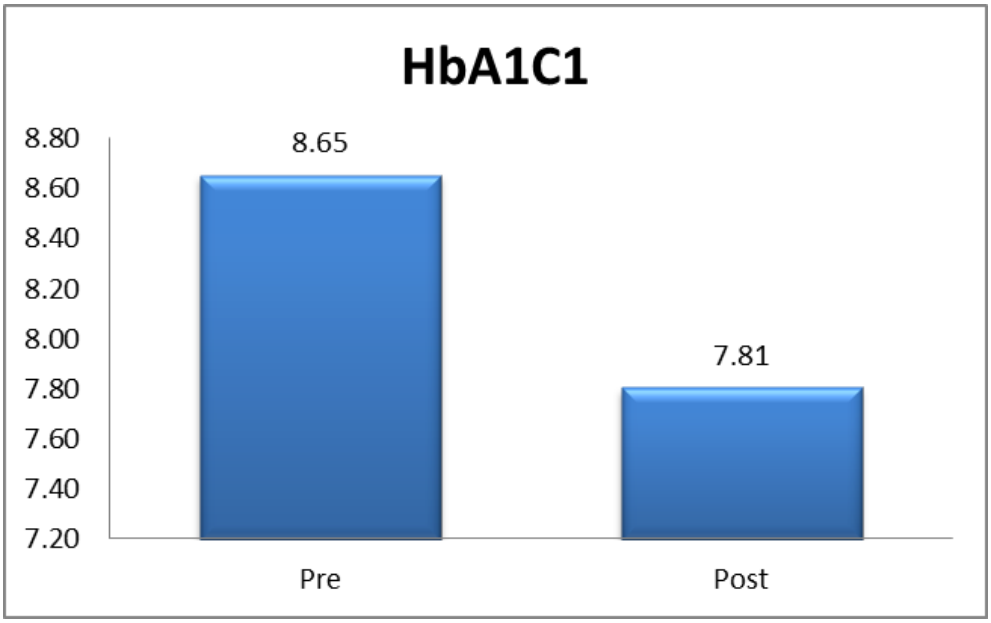


FIG. 43 - INITIAL AND FINAL VALUES – HBA1C

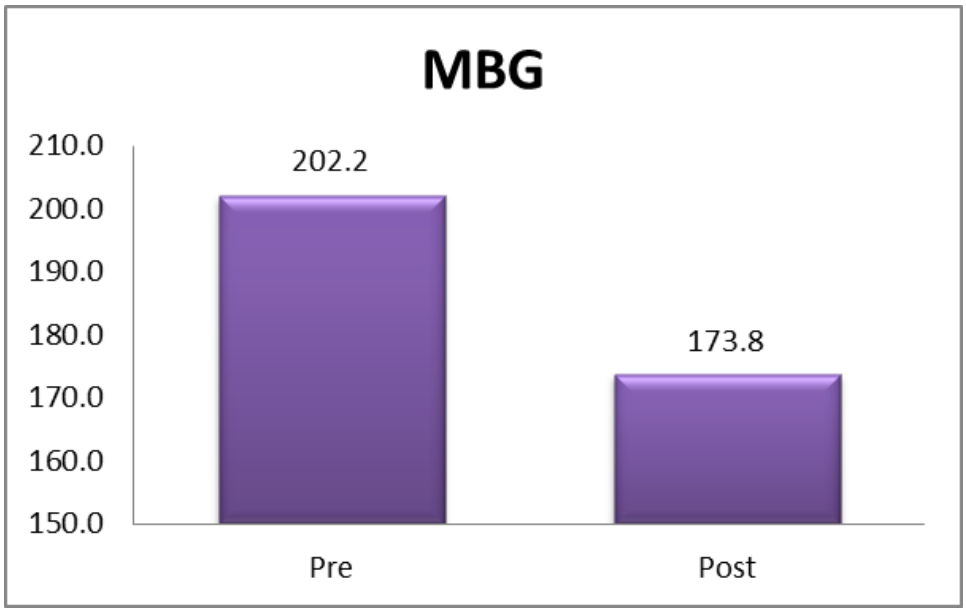


FIG. 44 - INITIAL AND FINAL VALUES – ESTIMATED AVERAGE GLUCOSE

The initial and final values of nail fructosamine in patients also indicate that glycemic control is achieved and was lower compared to initial values and hence the assay is comparable to other glycemic indices.

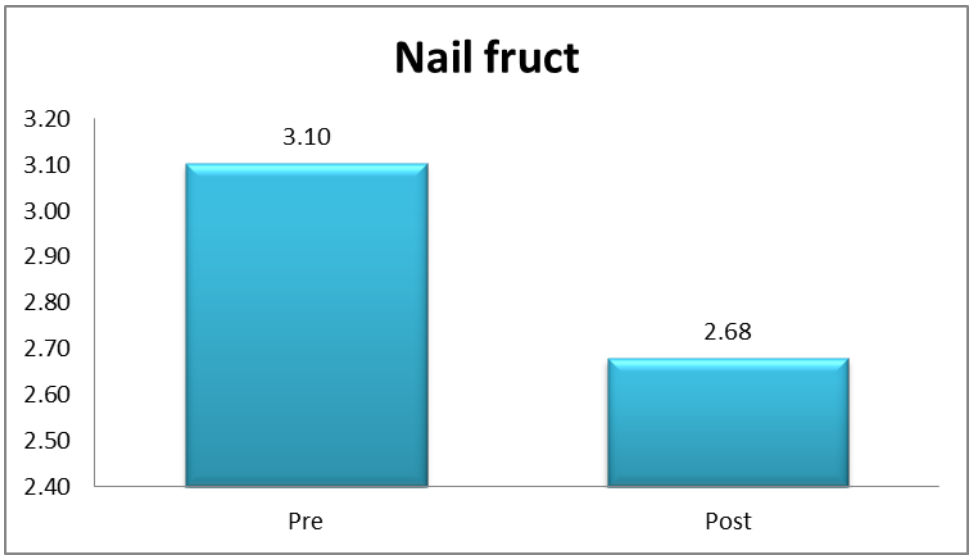


FIG.45- INITIAL AND FINAL VALUES – NAIL FRUCTOSAMINE

NAIL FRUCTOSAMINE VERSUS HbA1C:

The following figure show the linear relationship between nail fructosamine and Hemoglobin A1C.

FIG.46 - INITIAL AND FINAL VALUES NAIL& HBA1C COMPARED

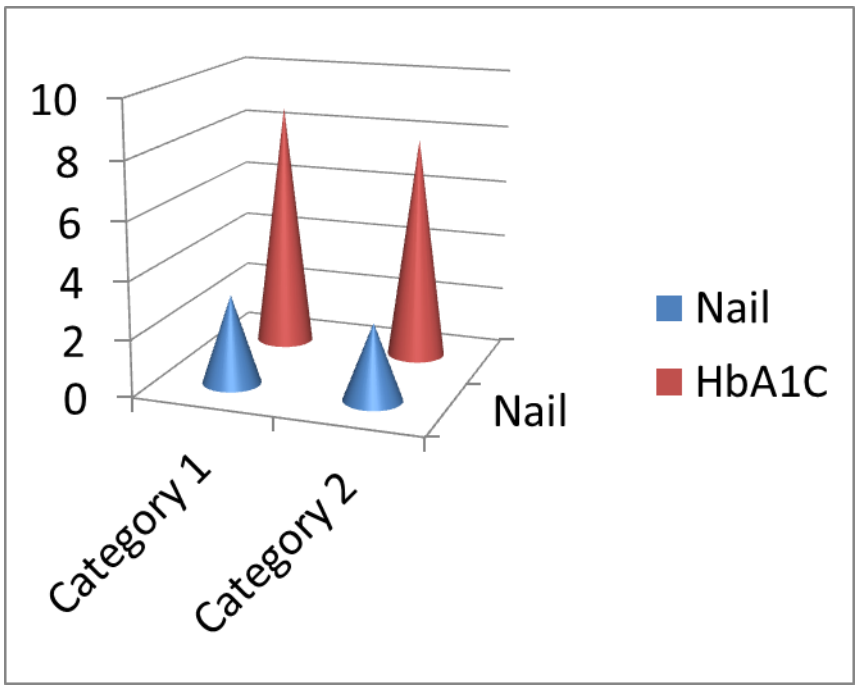


TABLE – 26 : PAIRED SAMPLES T- TEST:

VARIABLES		Mean	Std. Deviation	Std. Error Mean	TREND INITIAL - FINAL	t value	SIGNIFICANCE
INITIAL	HbA1C	8.654	2.1203	.3584	DECREASING	3.701	0.001
FINAL	HbA1C	7.81	1.480	.250			
INITIAL	MBG	202.17	70.742	11.958	DECREASING	3.721	0.001
FINAL	MBG	173.80	49.282	8.330			
INITIAL	Nail fructosamine	3.1017	1.39401	.23563	DECREASING	1.695	0.09
FINAL	Nail Fructosamine	2.6786	.90304	.15264			

HbA1C and nail fructosamine show significance and the values are 0.001 and 0.009 respectively.

PEARSON'S CORRELATION:

As per the aim of the study, the correlation of nail fructosamine to HemoglobinA1C is performed using PEARSON'S CORRELATION. The results are as follows:

TABLE : 27

Correlations

		HbA1C1	HbA1C2	Nailfruct1	NailFruct2
HbA1C1	Pearson Correlation	1	.772**	.068	-.107
	Sig. (2-tailed)		.000	.696	.541
HbA1C2	Pearson Correlation	.772**	1	.039	.038
	Sig. (2-tailed)	.000		.822	.831
Nailfruct1	Pearson Correlation	.068	.039	1	.229
	Sig. (2-tailed)	.696	.822		.186
NailFruct2	Pearson Correlation	-.107	.038	.229	1
	Sig. (2-tailed)	.541	.831	.186	

**. Correlation is significant at the 0.01 level (2-tailed).

The values are 0.068 and 0.038 for the initial and final set of values and weakly correlated.

COMPARISON OF INDIVIDUAL GROUPS - GLYCEMIC CONTROL

TABLE - 28 : HbA1C CUTOFFVALUE6.5%

HbA1C%	CONTROLS	PATIENTS	Total
<6.5	35	8	43
	100.0%	22.9%	61.4%
	0	27	27
>6.5			
	0.0%	77.1%	38.6%

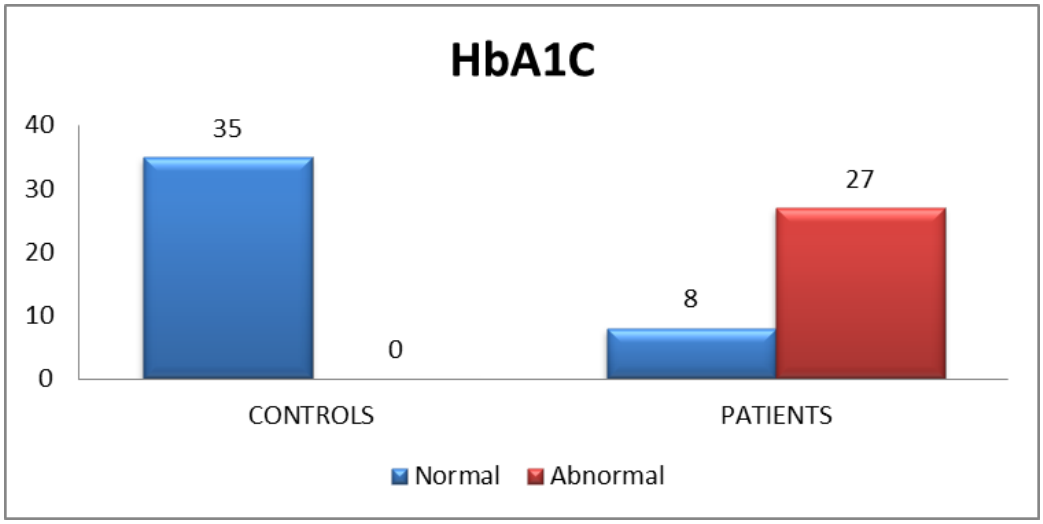


FIG.47 : HBA1C VALUES BELOW AND ABOVE CUTOFF VALUE – 6.5%

FREQUENCY BELOW AND ABOVE PHYSIOLOGICAL RANGE (2.5 µmol)

TABLE.29 : NAIL FRUCTOSAMINE

NAIL FRUCTOSAMINE µmol/g			
	CONTROLS	**PATIENTS**	**Total**
<2.5	24	18	42
	68.6%	51.4%	60.0%
>2.5	11	17	28
	31.4%	48.6%	40.0%

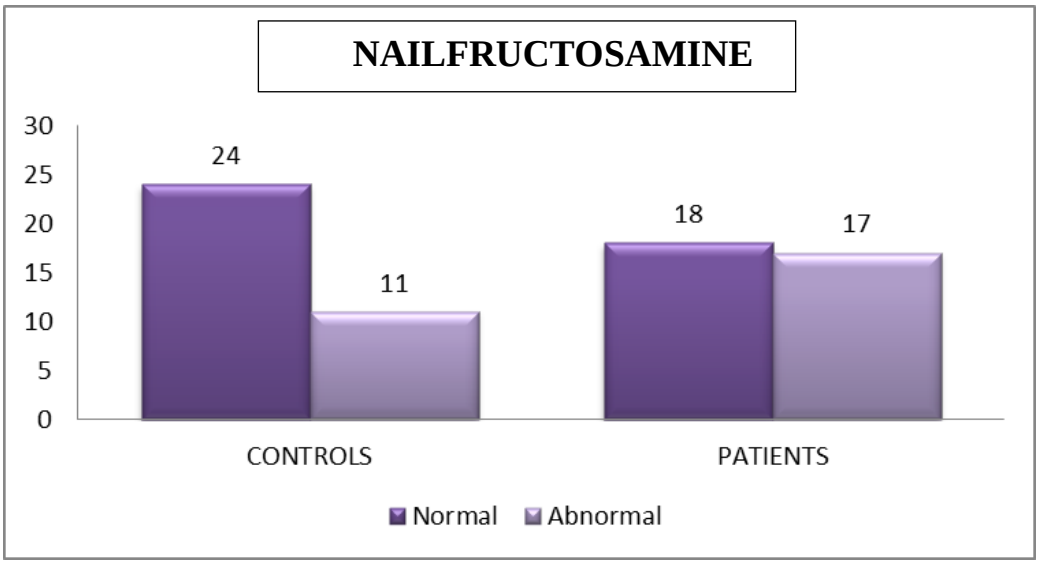


FIG : 48 - NAIL FRUCTOSAMINE BELOW AND ABOVE CUT OFF VALUE 2.5µMOL

TABLE – 30 : SERUM FRUCTOSAMINE:

SERUM FRUCTOSAMINE			
	CONTROLS	**PATIENTS**	**Total**
<285µmol	22	7	29
	62.9%	20.0%	41.4%
	13	28	41
>285µmol	37.1%	80.0%	58.6%

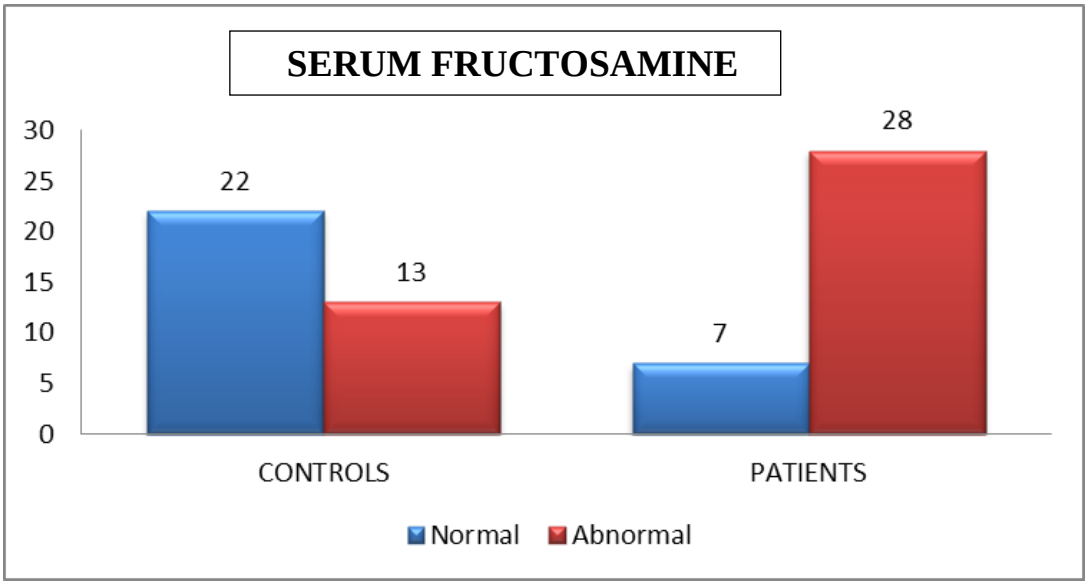


FIG.49 : SERUM FRUCTOSAMINE VALUES ABOVE AND BELOW CUT OFF 285µMOL

There is an overlap seen in the glycemic indices, more in nail fructosamine and serum fructosamine as seen in other studies.

RECEIVER OPERATING CHARECTERISTIC CURVE ANALYSIS:

NAIL FRUCTOSAMINE ASSAY:

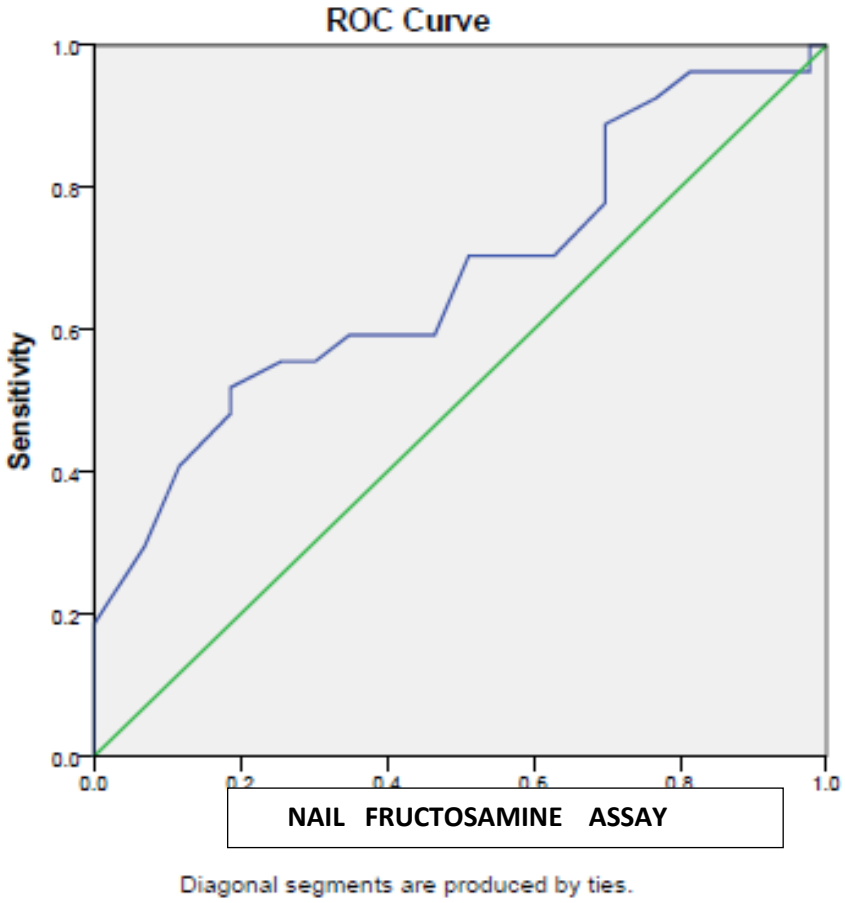


FIG.50 : RECEIVER OPERATING CHARECTERISTIC CURVE

Analysis of the ROC curve showed area under the curve of 0.674 (95%Confidence interval of 0.539 – 0.809 and a cut- off point of 2.35µmol/g equivalent of nail.) p value of 0.015) with a sensitivity 59.3% and specificity 58.1%

The individual values (56) of the two glycemic indices were comparable and show linear relationship except for four patients.

Two were hypothyroid, on eltroxin, one patient on treatment for schizophrenia and the other was taking aspirin for treatment of cerebro - vascular complication.

Thus an evaluation for confounding factors in the assay due to alcohol consumption, commonly used drugs and other substances will enlighten us on further implication of this assay.

REGRESSION ANALYSIS (LEAST SQUARES METHOD)

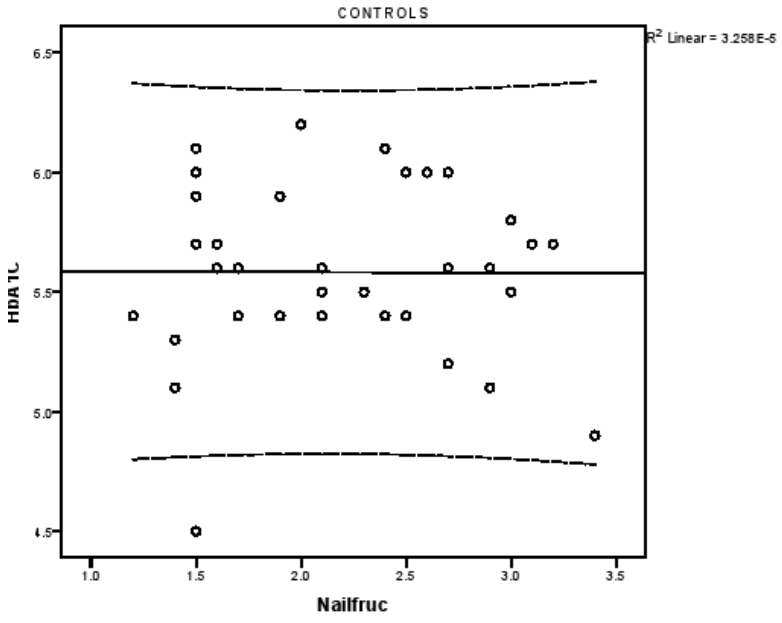


FIG.51 : CORRELATION – NAIL FRUCTOSAMINE & HBA1C

CONTROLS R VALUE >0.5 LINEAR

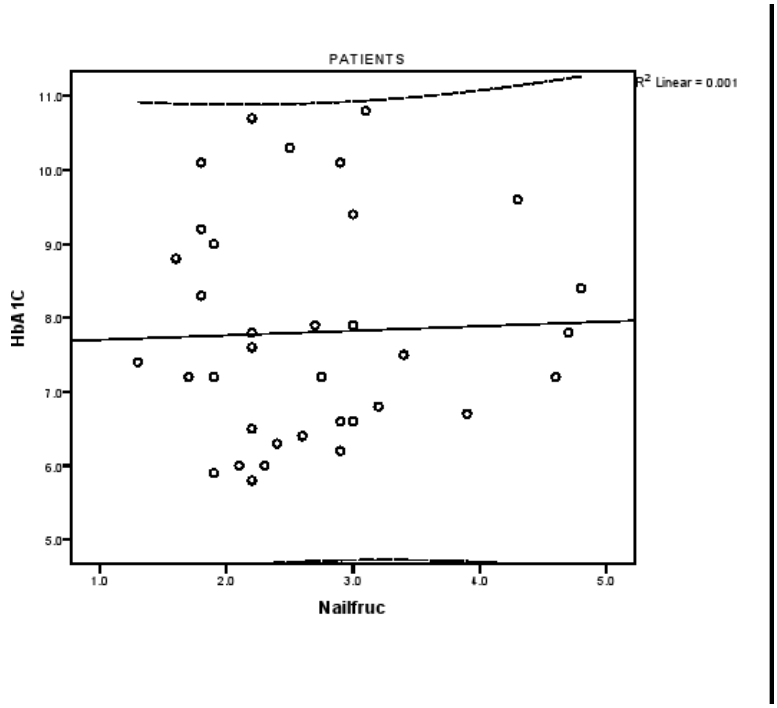


FIG. 52 : CORRELATION NAIL FRUCTOSAMINE AND HBA1C –CASES -LINEAR

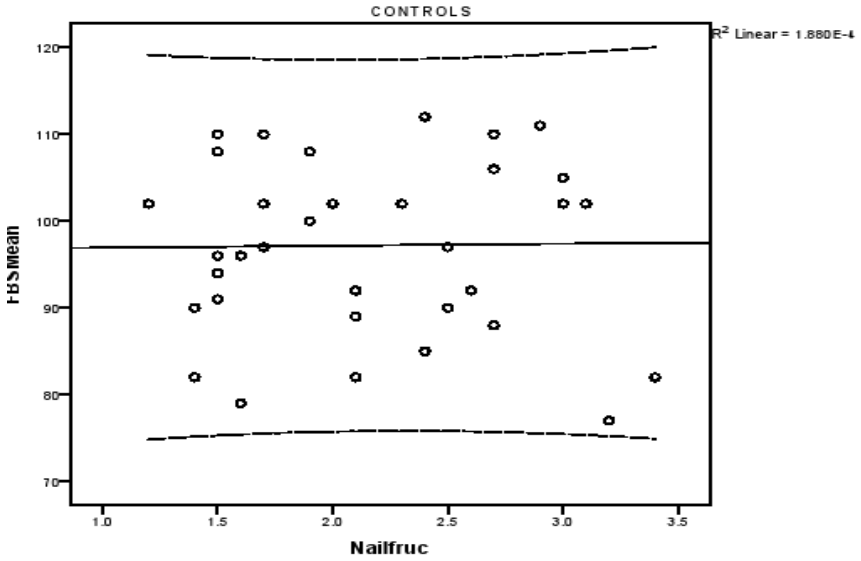


FIG.53 : CORRELATION NAIL FRUCTOSAMINE AND FASTING BLOOD GLUCOSE LINEAR - CONTROLS

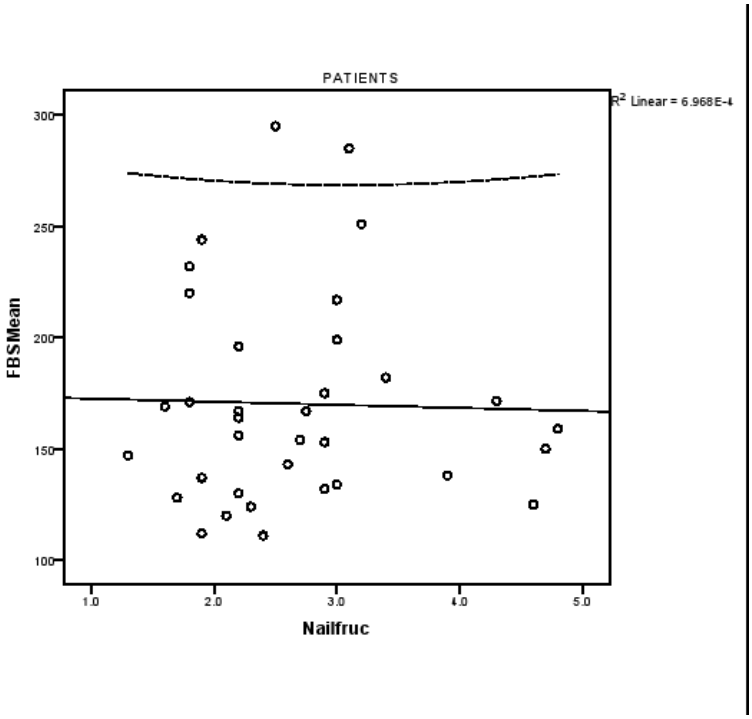


FIG.54 : CORRELATION GRAPH NAIL FRUCTOSAMINE AND FASTING BLOOD GLUCOSE – PATIENTS LINEAR

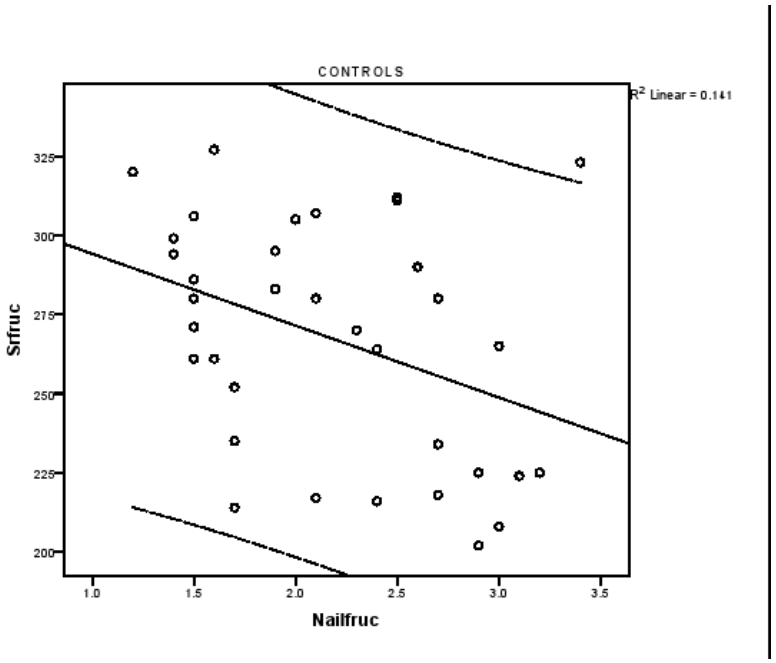


FIG .55 : NAIL FRUCTOSAMINE AND SERUM FRUCTOSAMINE – CONTROLS LINEAR.

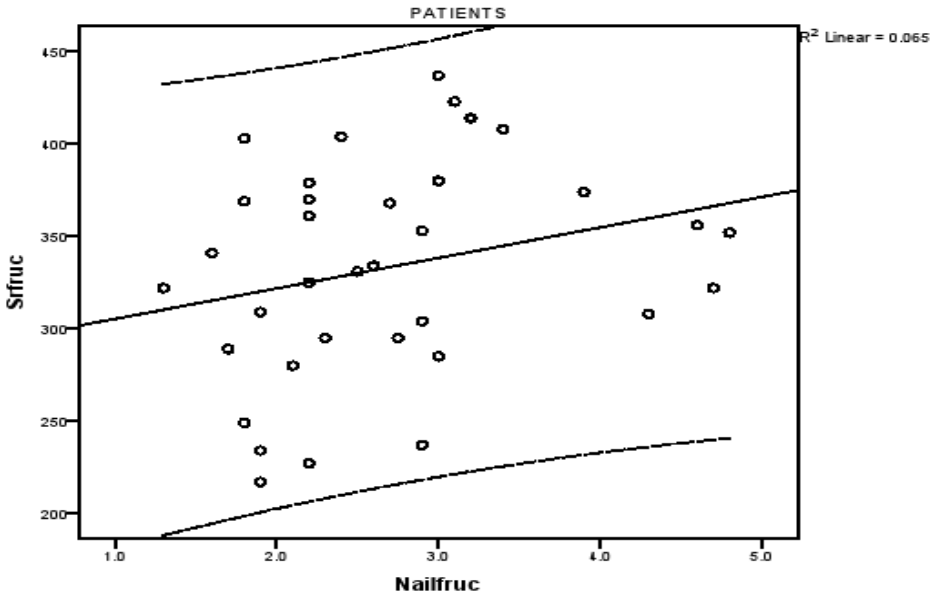


FIG.56 : NAIL FRUCTOSAMINE AND SERUM FRUCTOSAMINE – CASES - LINEAR

The r values are well correlating for nail fructosamine and serum fructosamine in patients and controls than for HbA1C and fasting blood glucose by the least squares regression analysis.

DISCUSSION

Analysis of the results :

1. The glycation in nail protein which forms 80% of the nail tissue depends on the blood glucose levels of the individual and reflects the average blood glucose over the preceding three months ie the duration of the nail growth across the nail plate. Hence it is compared with HbA1C which also reflects the average glucose over the same duration.
2. Out of the 70 individuals analyzed , 35 are known diabetics and 35 are healthy non-diabetic subjects of the same age group,whose Fasting Plasma Glucose is <126 mg %.

3. The observations made are:
   a) Glycemic status analysis.
   b) The risk factor analysis

      1. Glycemic status of the individuals is studied by comparison of Fasting blood Glucose, HbA1C, Serum Fructosamine with nail fructosamine for all patients and compared with control population.

2. The relationship of the nail fructosamine with FBS is linear.

3. The relationship of nail fructosamine and HbA1C is linear.

4. The relationship of nail fructosamine and serum fructosamine is linear.

5. As observed by A.S. Kishabongo et al (2013)(REF) the ROC analysis for nail fructosamine yielded a cut off value of 3.14 μmol/g of nail ,but in this assay ,it was found to be 2.35μmol/g of nail, the reason could be the smaller sample size.

6. The mean for diabetics and control population studied were 3.1 and 2.18μmol/g respectively in the initial readings taken .

7. As the aim of the study was to detect the glycemic control achieved the repeat values for the same individuals were done after 10 - 12 wks and the mean for the final values was 2.68μmol/g .This reflects the glycemic control achieved during the period of study. The same finding is

also confirmed by the mean values obtained in Hb A1C (figure 43 to 46) initial and final.

8. Both initial and final values in the nail fructosamine were found to be above the physiological range 2.0 - 2.5μ mol fructosamine / gm of protein, as observed by Goldsmith , whereas the control group mean lies within the physiological range.(2.18μ mol)

9. The mean values of controls as compared to the values given by Kishabongo et al (1.75 μmol) done in the sub-Saharan African population is less than that done in our south Indian population because the staple food varies between these two countries. The following figure clarifies this issue, the reasons being the staple cereal is maize in sub-Saharan region (glycemic index 55) but for the population in this study is rice (glycemic index varies from 86 -109 depending upon the variety of rice).

10.DATA FROM THE REPORT OF CCAFS
(Climate Change Agriculture and Food Security)

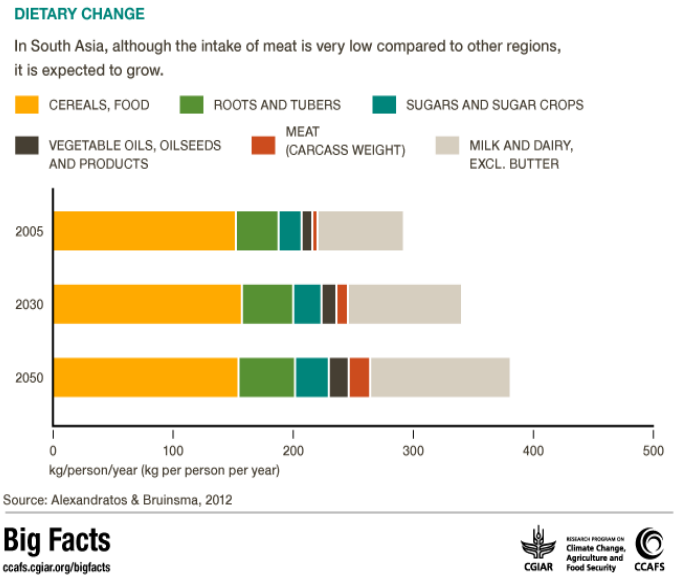


FIG.57 : FOOD CONSUMPTION DATA – SOUTH ASIA

The cereal consumption is more in proportion to the total food intake in south Asian population and rice being the staple food.

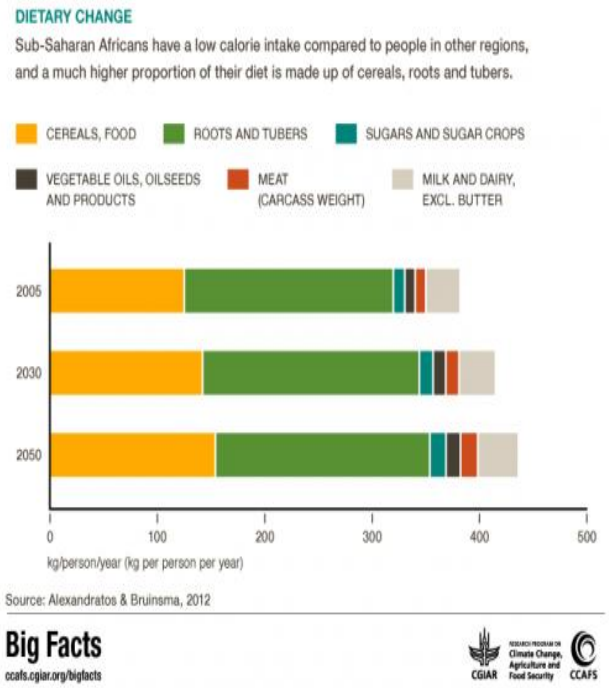


FIG.58 : SUB – SAHARAN AFRICA – DIET PATTERN

11. The cereal consumption is less in proportion to the total food intake. The roots and tubers taken in greater proportion are rich in fibre These food habits are possible reasons for the lower mean value for nail fructosamine in control population in the sub-Saharan region.

12. Also the population included for study as per the sub - Saharan study were aged 1 - 91 years and mean for controls is 36 yrs and for diabetics 62 yrs .Considering the median for diabetics without complications in the literature referred,it was 3.77μmols. In our study mean age for controls is 41.54 and cases is 51 yrs.

The values are less in our study group because of the difference in age and duration of the diabetes .

13. Both nephropathy and retinopathy were excluded from our study. The individuals with nephropathy and retinopathy were included in the sub Saharan study and their median nail fructosamine values were in the higher range 5.64 and 5.65μmol/g of nail.

4. The nail fructosamine assay is easier, simpler and cost effective.

5. The sample collection can be done by the patients themselves or by their relatives. Sample collection is easier and do not need technical personnel.
6. Transport and preservation of sample is easier.
7. The sample is stable for >3 weeks.
8. Pre-analytical variation is practically “NIL”
9. The sample could be collected non- invasively. The fear of needle prick alleviated and the pain can be avoided .It is useful for the patient’s long term follow up.
10. The procedure is simple to perform and can be performed in any clinical laboratory with basic equipments. (spectro photometer)
11. The precision of the assay is good.

CONCLUSION

On comparing with other glycemic indices like fasting plasma glucose, serum fructosamine and HbA1C, nail fructosamine shows linear relationship.

Interference in the assay due to commonly used drugs has to be evaluated and then the procedure can be standardized for routine use in the laboratory to assess glycemic status of the individuals both diabetic and non- diabetic individuals.

SUMMARY

A study was conducted in Government Stanley Medical College and Hospital. In the Department of Biochemistry, titled glycated nail protein as an indicator of long term glycemic control.

A total of 70 individuals, 35 known diabetic patients and 35 healthy individuals were enrolled for the study. The patients were longitudinally followed up for three months to assess their glycemic status.

The aim was to establish whether the nail fructosamine could be used to determine the glycemic status of the diabetic patients over the preceding three months which is the turnover time of the nail from root to the free edge.The Hemoglobin A1C estimation in the same patients at a same time was used to confirm the changes in the patient's glycemic status and compared with nail fructosamine. A linear relationship was established after analysis of the results.

The fasting blood glucose and serum fructosamine were also measured for assessing the blood glucose levels. These parameters on comparison with nail fructosamine showed linear relationship.

No interference was done in the treatment schedule of the patients.

Also the risk factors like positive family history, blood pressure, BMI, lipid profile, were also analysed. Results showed only moderate increase in risk factors among patients than controls.

To conclude the nail fructosamine assay could be standardized for testing the levels of blood glucose over the previous three month's duration.

The reference range in south Indian population needs to be established.

The interference like drugs and other dietary substances which might react with nitrobluetetrazolium need to be studied and the procedure can be used as routine laboratory investigations to assess the blood glucose levels on long term basis. The method is cheaper , easier to perform and sample collection and preservation is easier (nail clippings form finger nails).

Pre analytical variation of the analyze as observed with other analyzes is practically nil.

Cost of testing is less compared to other indices. Precision of the assay is good. CV Calculated was 7.8%

PATIENTS

SI. No	Age	Sex	Duration	Family H/o	Treatment	Comorbid	Height cm	Weight Kg	PR / mt	Bpmm Hg	Gr. Prturia	Ptnemia	Urine PCR	Urea	Creatinine	Cholester	TGL	HDL	LDL	VLDL	FBS Mean	MBG-1	HbA1C1	MBG-2	HbA1C-2	Nailfruct1	SrFruct1	NailFruct2	Protein	Alb	Hb%	BMI
1	66	F	7yrs	FMB	IN+OHA	Nil	156	66	82	150/70	Nil	Nil	0.1	18	0.4	250	208	40	168	42	171.5	224	9.3	234	9.6	2.27	308	4.3	5	3	10.3	27
2	49	M	5mo	nil	OHA	Nil	168	72.5	78	150/80	nil	nil	0.02	24	0.6	114	178	40	38	36	199	217	9.1	177	7.9	4.3	437	3	5	2.3	14.4	25.7
3	56	F	7mo	nil	OHA	Nil	159	60.4	82	140/70	Nil	Nil	0.02	16	0.9	186	126	58	103	26	159	194	8.4	194	8.4	2.6	352	4.8	5	3	12	23.9
4	50	F	3mo	Nil	OHA	Nil	158.5	62.7	72	110/60	Nil	Nil	0.07	24	1	166	104	62	83	20	143	170	7.7	127	6.4	3.2	334	2.6	7	3.5	12	25.1
5	52	M	3yrs	F	OHA	HTCVA	161	78.3	78	110/70	Nil	Nil	0.04	20	1.1	100	178	30	35	36	164	254	10.2	270	10.7	2.1	361	2.2	8	3.8	13	30.2
6	61	F	8yrs	FBGf	OHA	HT	153	42.6	92	120/60	Nil	Nil	0.05	24	0.9	206	128	44	134	56	128	147	7	154	7.2	1.5	289	1.7	7.5	3.1	11.3	18.2
7	48	F	0.6yrs	nil	OHA	Nil	158	65.6	86	130/80	Nil	Nil	0.025	24	0.8	172	368	44	41	70	285	294	11.4	274	10.8	1.6	423	3.1	8	3.5	11	26.3
8	40	F	1yr	F	IN+OHA	HypoTh	151	74	72	130/90	Nil	Nil	0.24	26	0.6	172	258	44	77	52	134	144	6.9	134	6.6	2.2	285	3	8	2.8	10.8	32.4
9	49	F	4yrs	FM	IN+OHA	Nil	143	53.6	86	120/80	Nil	Nil	0.05	20	0.4	165	134	38	264	101	295	334	12.6	257	10.3	4.2	331	2.5	7.8	4.2	11.7	26.3
10	43	F	12yrs	BS	OHA	Nil	146	42.3	82	110/70	Nil	Nil	0.02	28	1	260	144	52	184	28	182	207	8.8	164	7.5	3.8	408	3.4	5.1	3.1	9.2	19.9
11	61	F	10yrs	M	OHA	HypoTh	160	84	76	120/70	nil	Nil	0.12	28	0.3	212	123	26	160	28	244	260	10.4	214	9	7.5	217	1.9	8.2	3.3	13.4	32.9
12	67	M	22yrs	F	OHA	nil	167	61	82	130/90	nil	Nil	0.09	30	0.6	204	156	46	127	28	156	180	8	167	7.6	4.4	370	2.2	8.3	4.6	12.8	21.9
13	67	F	12yrs	nil	OHA	nil	148	59.3	70	90/60	Nil	Nil	0.1	32	0.8	178	100	54	138	20	112	204	8.7	154	7.2	2	309	1.9	8	4.8	11.6	27.1
14	45	F	4yrs	Gm	OHA	PE	159.5	73.3	88	120/70	Nil	Nil	0.093	26	0.5	198	146	22	145	30	217	270	10.7	227	9.4	4.9	380	3	7.5	3	12.4	29
15	45	F	4yrs	B	OHA	HypoTh	153	71.3	72	100/70	Nil	Nil	0.16	24	0.8	118	156	22	66	28	167	157	7.3	130	6.5	2.74	227	2.2	5.9	3	10.7	30.5
16	63	F	12yrs	nil	OHA	OA	143.5	65.9	74	120/60	Nil	Nil	0.05	20	0.9	286	144	42	212	28	138	167	7.6	137	6.7	4.4	374	3.9	6.7	3.8	11.5	31.8
17	51	F	1Yr	M	OHA	PT	143	60	96	130/80	nil	nil	0.08	25	1	168	142	30	91	30	169	250	10.1	207	8.8	2.5	341	1.6	8	4.5	13.4	29.4
18	40	F	1.5yrs	M	OHA	nil	150	71	82	130/80	nil	nil	0.03	31	1	196	180	42	118	36	147	177	7.9	160	7.4	1.4	322	1.2	7.9	4.3	13.1	31.5
19	40	F	3yrs	C	oha	nil	165.5	8.5	80	120/80	nil	nil	0.05	30	0.8	140	265	38	49	54	232	284	11.1	250	10.1	2.7	403	1.8	7.7	3.9	9	30.9
20	50	F	2yrs	nil	OHA	HT	152	59	72	130/80	Nil	Nil	0.03	24	0.9	280	298	44	86	60	171	197	8.5	190	8.3	2.1	249	1.8	5.1	2.9	9.4	25.5
21	42	F	1 yr	M	OHA	HT,OA	145	72	84	130/80	Nil	Nil	0.04	20	0.9	140	256	38	52	52	120	117	6.1	114	6	1.6	280	2.1	6.5	3	12	34.3
22	55	M	6yrs	F	OHA	Nil	156	48	80	110/70	Nil	Nil	0.07	23	0.6	210	315	20	130	63	132	124	6.3	120	6.2	6.2	237	2.9	7.7	3	14.1	19.8
23	55	F	1.5yrs	F,D	OHA	Nil	146	55	82	110/70	Nil	Nil	0.5	28	1.1	244	130	42	176	26	130	130	6.5	107	5.8	3.8	325	2.2	8	4.5	10.9	25.8
24	57	F	6yrs	nil	OHA	HT	146	67	82	110/70	Nil	Nil	0.052	35	1.2	150	136	22	100	28	220	274	10.8	220	9.2	2.5	369	1.8	7.8	4.7	12.8	31.5
25	34	M	0.5yrs	F	OHA	Nil	155	69	96	130/80	Nil	Nil	0.5	24	0.7	174	134	42	105	26	175	294	11.4	250	10.1	3.7	353	2.9	5	3.3	12.5	28.8
26	38	M	0.5yrs	SB	OHA	Nil	164	58	86	130/80	Nil	Nil	0.03	20	0.8	214	105	46	147	28	196	417	15.1	174	7.8	2.3	379	2.2	5.3	2.7	12.8	21.6
27	69	F	6yrs	S	OHA	HT, CAD	160	64	84	140/80	Nil	Nil	0.05	26	0.6	204	160	36	116	52	150	157	7.3	170	7.8	2.7	322	4.7	7	3.5	11.2	25
28	47	F	0.5 yrs	B	OHA	Nil	154	63	84	110/80	Nil	Nil	0.4	20	0.8	218	156	30	157	32	124	120	6.2	114	6	1.4	295	2.3	6	4	12	26.6
29	57	M	0.5yrs	nil	OHA	Nil	155	60	72	110/70	Nil	Nil	0.36	26	0.9	256	120	39	193	40	137	117	6.1	110	5.9	2.7	234	1.9	6	3.2	13.2	25
30	22	F	0.5 yrs	B	OHA	Nil	140	65	82	120/70	Nil	Nil	0.02	24	0.7	300	196	44	216	40	251	220	9.2	140	6.8	2.8	414	3.2	5.5	3	10.4	33.1
31	66	M	4yrs	nil	OHA	HT	161	64	80	140/90	Nil	Nil	0.03	18	0.6	210	261	34	124	52	153	140	6.8	134	6.6	3	304	2.9	5.3	3	12.3	24.7
32	61	F	6yrs	nil	OHA	Nil	150	58	86	130/80	Nil	Nil	0.05	22	0.6	298	158	39	228	48	125	147	7	154	7.2	5.5	356	4.6	7.5	3.2	13	25.8
33	42	M	4yrs	F	OHA	Nil	153	58	84	130/80	Nil	Nil	0.01	25	0.8	176	190	38	102	38	154	224	9.3	177	7.9	3	368	2.7	6	3.6	12.5	24.8

34	47	F	7yrs	F	OHA	PN	146	63	74	120/70	Nil	Nil	0.03	29	1	166	178	36	95	36	167	140	6.8	154	7.2	2.65	295	2.75	5.3	3	9	29.6
35	54	F	0.5yrs	nil	OHA	Nil	146	54	76	120/70	Nil	Nil	0.02	30	1	130	156	40	60	32	111	124	6.3	124	6.3	2.3	404	2.4	5	3.5	11.5	25.4

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PROFORMA

HbA1C Vs NAIL FRUCTOSAMINE
Department of Biochemistry Stanley Medical College, Chennai.

Name: Age: Sex: ID No: Date:

OP No: Type of Diabetes:
Positive Family History

Address: Duration: Type:

Drugs:

Metformin: Glipizide: Glibenclamide:

Others:

Insulin dose: Duration :

Co-morbid illness:

HT/CAD/ Nephropathy/Neuropathy/Thyroid disorders/ Cataract/Surgeries underwent

Examination:
General PR BP System:
CVS: RS: CNS: ABD:
Others:
Diagnosis:
Routine Investigations:
Urine: Alb: Sug: Creat:
Blood: Hb% Cell count:
Urea: Creat: Gluc: Protein:
Alb: A/C ratio
Chol: TGL HDL ECG:

HbA1C	MBG	FBSM	SFM	NF

S.No	Date	HbA1C	MBG	FBG-M	SF	NF
I						
II						
III						
IV						

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