THROMBOPHILIA IN PERTHES' DISEASE

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT TO THE M.G.R MEDICAL UNIVERSITY FOR THE DEGREE OF MASTER OF SURGERY IN ORTHOPAEDICS.(2005-2007)

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CERTIFICATE

This is to certify that this dissertation entitled **"THROMBOPHILIA IN PERTHES' DISEASE."** is the bonafide work of **Dr. Vinu Mathew George.** This study was undertaken at the Department of Orthopaedic and Accident Surgery, Christian Medical College and Hospital, Vellore, Tamil Nadu, in partial fulfillment of the requirement of the award of Degree of **M S in Orthopaedic Surgery** of Dr. MGR Medical University, under my guidance.

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I hereby declare that this dissertation entitled **" THROMBOPHILIA IN PERTHES' DISEASE."** is original work done by me under the guidance of **Professor Vrisha Madhuri,** Department of Orthopaedics and Accident Surgery, Christian Medical College and Hospital, Vellore, Tamilnadu. This has not been submitted to any other University or Board of examinations, in part or full.

Place: Vellore. **Dr. Vinu Mathew George**

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II. - INTRODUCTION

LEGG-CALVE-PERTHES' DISEASE
"What's in a name? that which we call a rose
By any other name would smell as sweet."
Romeo and Juliet, 11. 43
Quoted by Perthes', 1920.

II.i Rationale for the study

Legg-Calvé-Perthes' syndrome is a disorder involving the hip in children that results from an interruption to the blood supply of the capital femoral epiphysis. The disorder was described independently by Legg, Calvé, Perthes and Waldenstrom in the early twentieth century. Perthes was the first to describe the pathology in which he found cartilage islands with no features of infection or inflammation. Sundt described the 'susceptible child'. He believed that people who were so predisposed would develop the disease once they sustained trauma to the hip.

'Trauma' here is generically used to imply an insult to the hip that tips the balance causing the disorder and included either injury or infection.

There have been recent studies implying that there are prothrombotic tendencies in patients with Perthes' disease, especially in western populations. But other studies have refuted this association. The existing literature has implicated the following factors:- Protein C (PrC), Protein S (PrS), Factor V Leiden mutation (FVL), Hypofibrinolysis, Low levels of Antithrombin III (AT)([1,2,3,4,5,6,7,8,9])

In our hospital, more than 50% of our patients with Perthes' disease come from West Bengal; we hypothesise that there is a locus of disease there similar to the already proven loci in Liverpool, the Konkan region and Kerala. A population-based study is required to prove this conclusively.

A previous audit of our patients revealed three sets of brothers with Perthes' disease. This led us to consider that, at least in some patients, there was probably a genetic or hereditary basis for disease. In addition, our patients predominantly had severe disease and two had recurrent Perthes' disease suggesting repeated insults. These observations are consistent with a thrombophilic etiology which has been proposed in the last decade. Literature reveals many studies where evaluation of some thrombotic traits was done, but it was not comprehensive and the findings of various studies are in conflict with each other. We made a comprehensive list of thrombotic factors that could be evaluated at our institution. These include all known factors with the exclusion of Thrombin-activable fibrinolysis inhibitor (TAFI).

There is a report of more severe disease being prevalent in patients with a homozygous mutation for Factor V Leiden gene.[10] The incidence of the F V Leiden mutation in India is much lower

than in the West (data from our clinical pathology lab, 2004-5 on patients who underwent the thrombotic work up for DVT) and Ghosh et al. who reported that an incidence of only 3% of patients with deep vein thrombosis (DVT) had the F V Leiden mutation in a prospective study of 432 patients from western India. They reported that at least 34% of them had a demonstrable cause for thrombophilia. In a study of patients with hepatic venous outflow tract obstruction (HVOTO) Kumar et al reported a low allele frequency of FVL mutation - 2% in controls and 3.4% in cases, in their study population of 59 patients. [11, 12]. This is opposed to the high incidence of Factor V Leiden mutation reported in western English literature where it was found to be present in up to 14.5% [13]

Extrapolating this, i.e. the severity of Perthes' disease and presence of thrombophilia is linked, and that the prevalence of thrombophilic factors are different in the Indian and western populations, we assumed that alternative thrombotic tendencies were present in our population. So we modified the inclusion criteria to include patients with more severe disease and those with a family history of disease to increase the proportion of patients with thrombophilia in our study population. If this picked up a large incidence of patients with thrombophilia, we planned to extend this study to include all patients with Perthes' disease. In Perthes' disease, the focus has been on the treatment of the avascular necrosis after the damage has occurred. The study of thrombophilic etiology has an attraction for the orthopaedic surgeon as it opens up the possibility of preventive treatment.

There are no studies in India delineating the baseline values for thrombosis. Furthermore, there are no studies on the subset of Perthes' disease patients. Ours is a pilot study in children with Perthes' to see if they have a prothrombotic tendency.

II.ii. Aim

To investigate the role of thrombophilia in the etiology of Perthes' disease in India.

II.iii. Objectives

1. To study the genetic mutations associated with thrombosis in Perthes' disease.
2. To evaluate prothrombotic tendencies among children with Perthes' disease.
3. To geographically map the patients who formed the study population.

III. REVIEW OF LITERATURE

It is impossible to answer the question regarding etiology."

Calve, Jacques, Sur une forme particuliere de pseudo-coxalgie greffee sur des deformations caracteristiques de l'extremite superieure du femur, Revue de Chirurgie 30:54-84, 1910. (English translation) EX1

When a thing ceases to be a subject of controversy it ceases to be a subject of interest.

William Hazlitt, English essayist and critic, 18th century.

III.i. History

Tuberculosis reached epidemic proportions during the 19th century in the industrialized countries. Bone and joint tuberculosis was common, and its treatment formed a major part of the contemporary surgeons' work. Occasionally, however, it was noted that children who were thought to have 'tuberculous hip disease' recovered quickly and with excellent function. Hugh Owen Thomas constructed a device for the treatment of this form of disease which survives to today as 'the patten-ended caliper', and is still used by some in the treatment of Perthes' disease.

The treatment of choice for this 'nonsuppurative' type of hip disease was excision of the femoral head. This was advocated mainly by German surgeons and remained 'text-book teaching' until 1902. Noble Smith, in London, thought that because not ***every*** case of hip disease had tuberculosis as its origin, excision of the femoral head should not be performed as routine treatment.

In 1895, in Wurzburg, Professor Wilhelm Roentgen presented his discovery: "Uber eine Art von Strahlen" (On a New Form of Rays) at the meeting of the local Physicomedical Society. His discovery changed orthopaedics and, in the process, allowed Perthes' disease to be differentiated from tuberculosis on radiological grounds.

Alban Kohler, the world's first professor of radiology, in Wiesbaden, in 1905, presented a case of flattening and later fragmentation of the femoral capital epiphysis in a child, which he thought did not have tuberculosis as its origin. It reminded him of an infarct *(infarctiihnlich).* He repeated this observation in 1908 when he described the condition affecting the tarsal navicular, which still bears his name.

Calvé noted a special type of 'coxalgie' i.e. 'pain in the hip' in which severe radiological changes did not correspond with the minimal clinical signs. He called the condition "coxalgie fruste" and drew the attention of his friend Sourdat to this phenomenon who published his account in 1910, acknowledging Calvé's work. In 1909, Henning Waldenstrom, later to become professor of orthopaedics at Stockholm, gave an account of seven of his own and three of Sindig-Larsen's patients. The children reported in this paper had developed an intermittent limp with little pain and no systemic upset. The observed radiological changes were suggested to have been brought about by tuberculosis of the hip interfering with the blood supply of the hip. Though Waldenstrom thought that the disease

was a form of tuberculosis, he concluded: “I have presented here the cause, origin and development of a perhaps quite common disease, which, however, has not been described before.”

At the meeting of the American Orthopaedic Association at Hartford, Connecticut, in June 1909, Arthur T. Legg of Children’s hospital, Boston, presented case histories of five children. His paper was published the following year. He thought that this “obscure affection of the hip joint” was caused by trauma, disrupting the blood supply to the femoral head by a minor displacement of the epiphysis. Such an injury would cause a hyperemia of the neck of the femur, resulting in the thickening seen on the radiographs.

In 1910, Jacques Calvé an assistant surgeon at Berck, presented his ‘Sur une forme particuliere de pseudocoxalgie’ [14] where he gave an account of 10 patients in whom they found no sign of congenital syphilis. One of his patients had both hips affected, and two were brother and sister. He considered the cause to be an abnormal or retarded osteogenesis, such as that brought about by rickets. He felt that a low-grade infection in a thus affected hip could cause the radiologically observed changes. He thought that this condition was not tuberculous in origin and did not correspond with any previously described hip disorder.

Georg Clemens Perthes was professor of surgery at Leipzig, when in 1910, he presented his paper entitled ‘Uber Arthritis Deformans Juvenilis.’ In this detailed study, he presented 38 patients, 12 of them with bilateral disease. He summarized the clinical picture as well as the radiological appearances. He thought that the condition was not tuberculosis, and trauma did not play a major part in its etiology. Perthes thought that Kohler’s patients in 1905 probably had the same condition as the patients in his own paper. At this stage, he thought that the disorder came about as a consequence of an incompletely healed hip joint inflammation in infancy. By 1913, Perthes revised his views on the condition, which he then called “osteochondritis deformans juvenilis.” He presented six more case histories and brought together the classical clinical and radiological phenomena of this ailment, which still hold largely true today. Perthes obtained a biopsy specimen from one of the patients and found no inflammatory response in the synovium or in the segment from the femoral head. Marrow and bone necrosis were present with intermingling of small cartilage islets. He referred to Lexer’s work when he formulated his new theory of the etiology: the disorder might be due to interference with the blood

supply of the femoral capital epiphysis, the cause of which is unknown. It was because of Perthes' contributions that the condition soon came to be called Perthes' disease.

Other observers thought that the occasional familial occurrence of the disorder was difficult to reconcile with the theory of localized vascular disturbance at the upper end of the femur. Thus the discussion on whether Perthes' disease was inherited or not is about a century old!

In 1915 Allison and Moody tried and failed to reproduce the condition in rabbits by interfering with the blood supply to the femoral head; the reason why it is difficult to reproduce in animals is that they do not bear weight on the affected limb and therefore collapse does not occur.

The 1920s saw an enormous number of publications on Perthes' disease. This was the era of "osteochondritismanship" (Mercer Rang), when osteochondritis of virtually every growing epiphysis was 'discovered'. The term 'osteochondritis' was originally coined to describe the changes seen in congenital syphilis. Perthes believed that several different causes could lead to the obstruction of arterial blood flow to the affected femoral head. He and other contemporary surgeons thought that the ailment could arise from several different etiologic factors operating in different individuals. [15,16].

III.ii. Epidemiology

III.ii.a Incidence

The epidemiology of the disease in India has largely been limited to the studies done in western India. Joseph et al reported the incidence of Perthes' disease in Vellore taluk to be 0.4/100,000 per year in the 5-14 year age group (data provided from the Christian Medical College, Vellore) and 4.4/100,000 in Udupi taluk in the same age group. [17]

Barker et al., in 1978, reported on the incidence of Perthes' disease in three regions of England. They found that the incidence in the Mersey region (11.1 per 100 000 children under fifteen years) was twice that in Wessex *(5.5)* with Trent having an intermediate incidence (7.6). [18] Pillai et al in 2005 reported that in 40 children with Perthes' disease from South West Scotland (Dumfries and Galloway) the mean annual incidence of Perthes' disease was 15.39 per 100 000 children aged 0 to 14 years. [19] Hall and Barker in 1989 reported that the average annual incidence was 10.2 per 100,000 in boys aged 14 years and under and 2.2 in girls. [20]

The data given below, taken from the article by Hall and Barker [21] on the epidemiology of Perthes' disease and other articles show the highest incidence in Liverpool and ranges of incidence between 5 – 15/100,000 per year elsewhere. [22]. [23]

	Incidence		
Area	*Boys*	*Girls*	*Both Sexes*
Massachusetts[22]	—	—	5.7
British Columbia, Canada[14]	8.4	1.6	5.1
England[f]	12.4	3.3	8.0
Mersey Health Region	16.9	5.0	11.1
Trent Health Region	12.0	3.0	7.6
Wessex Health Region	8.7	2.0	5.5
Liverpool[16]	25.8	4.9	15.6
Eastern Cape Region,[26] South Africa (Whites)	—	—	10.8

III.ii.b Boy :Girl Ratio

The ratio of male to female involvement was 2.6:1 in combined cases from the data obtained from England and in the Indian population (unpublished data from areas in India as well as the United Kingdom as presented by Joseph et al) and 6.6:1 in the patients from Udipi taluk alone. The overall male:female ratio in 165 cases reported by Chacko et al in South India in 1986 was 2.58:1, with profound regional differences. [24] [17]

Wynne-Davies and Gormley, in 1978, found the ratio of males to females in Edinburgh and Glasgow to be between 4 to 5:1 in their study of 310 patients. [25] Barker et al had an average male to female ratio of 3.75:1 with ranges varying from 3.4 to 4.4:1 in their study of three areas- 72 came from Mersey, 78 from Trent and 34 from Wessex - of the UK, a total of 180 patients. [18] Pillai et al in 2005 reported that in 40 children with Perthes' disease the Male:Female ratio was 3.4:1 (31 boys and nine girls). [19] Hall and Barker in 1989 reported on a total of 101 children newly diagnosed as having Perthes' disease in Yorkshire. In their study, there were 84 boys and 17 girls, aged from 2 to 13 years showing a male female ratio of almost 5:1. [20] Most other studies have a ratio of around 4 or 5 :1 [23], [22]

III.ii.c Distribution

There are studies showing that there is an increased incidence of Perthes' disease in the urban population as compared to the rural population. A survey of cases of the disease in Liverpool, England, during six years (1976-81), showed a remarkably steep social class gradient, from 4 per 100,000 in Social Class I to 26 per 100,000 in Social Class V, mainly in the inner cities. [21] Conversely, the population of urban children affected by the disease in two cities in South India was much less than the rural population affected in the study by Joseph et al. [17,18.]

Pillai et al in 2005, reported on the spread of Perthes' disease in 40 patients from Dumfries and Galloway in Southwest Scotland who were identified by a retrospective review over a period of ten years from 1992 to 2002. There was a direct association between the incidence of the disease and deprivation scores, with the highest incidence in the most deprived areas**.** The incidence of Perthes' disease in rural Scotland is comparable with that in urban areas (15.4 per 100 000). [19] Hall and Barker in 1989, in their study of 101 patients, showed the rural urban distribution is as given below - wholly

urban - 62 , predominantly urban-27, mixed urban-6, mixed and predominantly rural-0, and wholly rural - 6 which shows a 62% localization in the urban population. They also reported that there was a steep rise in incidence from social class I to V. [20]

III.ii.d Age

In 165 cases of Perthes' disease observed by Chacko et al in South India in a 15-year period, the mean age at onset of symptoms was 9.89 years for boys and 8.71 years for girls. [24] Joseph et al in their study of Perthes' disease in South India mentions that the mean age of patients in India was approximately 10 years as compared to 6 years on average in the UK and the US. They also mentioned that this was unlikely to be explained by the fact that cases may be picked up later in India than in the west as the age distribution of Indian patients fit the log normal curve. The possible explanations given for this later onset of disease was that the aetiological factors may act at a later age in Indian children or that the time to onset to disease was longer in Indians. [18,17] . Pillai et al in 2005 reported that in 40 children with Perthes' disease from South West Scotland, the mean age at diagnosis was 6.5 years. [19] In the study by Gleuck et al in Cincinnati, the mean age of the children when Perthes' disease was first diagnosed was 5.8 ± 2.7 years. [1] Thus the mean age at presentation in Indian children was about 8 years and in other populations about 6 years.[25]

‘

III.iii. Etiology and Pathogenesis:

Perthes' disease is an avascular necrosis of the head of the femur in children. Various etiologies have been proposed for this. Since our study focuses on the thrombotic etiology for Perthes' disease, this will be discussed in more detail.

III.iii.a Histopathology

In the study by Inoue et al, the histological appearance of 57 femoral head biopsy specimens in Perthes' disease was studied. In 51 per cent of hips, histopathological changes characteristic of double infarction were present and there were grounds for postulating that double infarction might eventually occur in all cases. The findings support the concept that the deformation of the femoral head and the chronicity of Perthes' disease in man may be due at least as much or even more to repeated episodes of infarction and the ensuing abnormalities of growth rather than mechanical factors related to weight-bearing. In this study, they also examined canine femoral heads that were infarcted once, as well as, twice (after an interval of four weeks) and the human biopsies.

In those dogs, where the femoral heads were examined after four weeks, there was evidence of infarction and revascularization. There were areas of living woven bone deposited on a scaffold of dead bone and the marrow spaces were occupied by granulation tissue. After the second infarct, these attempts at repair also died and showed a characteristic and diagnostic picture of two infarcts: there was dead woven bone superimposed on dead lamellar bone, and the marrow space was occupied by dead granulation tissue. The histological changes in Perthes' disease are most prominent in the deepest layers of the epiphyseal cartilage as this is the zone that depends most on the epiphyseal blood supply and therefore most affected by the ischaemic process. The superficial layers of the cartilage are nourished by the synovial fluid. The bone, also supplied by the vessels, becomes ischaemic. In the human specimens, all the bone from which the ossific nucleus was originally composed of was infarcted i.e., every hip biopsied showed histological evidence of at least one infarct **throughout** its extent.

They found definite pathological evidence to link duration of symptoms and the incidence of double infarcts i.e. as the duration of symptoms increased, the number of specimens showing double infarcts also increased. Of the 23 hips with symptoms present for three months or less, 11 (48 per cent)

had pathological evidence of two infarcts. Of the 15 hips presenting at four to six months after the onset of symptoms, 10 (67 %) had evidence of two infarcts.

The basal layer of cartilage though, continues to proliferate but the ossification is deficient. This leads to the formation of tongues of cartilage extending to the metaphysis as the bone growth in the adjoining areas continues normally. As the ossific nucleus again became revascularised, these features diagnostic of two infarcts ultimately disappeared and the bone remodeled. . [26] [16]

III.iii.b Blood supply to the femoral head

The need for a definite understanding of the vascular structures during growth is important as it is believed that Perthes' disease is of vascular origin. First, the normal anatomy will be discussed and then the theories behind vascular and venous disease and how this is thought to cause Perthes' disease will be elaborated.

Langer, in 1876, showed by injection that vessels did, in fact, enter the developing femoral head through the ligamentum teres and that they were of fundamental importance to the epiphysis. He claimed that variations existed in the adult, but that these were secondary changes in which the vessels of the round ligament shrank to unimportance so that cervical vessels took over an almost exclusive supply of the head. Walmsley in 1915, examined 100 round ligaments but did not find a vessel of any size; he concluded that arteries of the ligament could convey no more than a small amount of blood. Furthermore, he demonstrated by injection that these vessels did not supply the ossific centre in two children aged two years and six years. Chandler and Kreuscher in 1932, examined 114 round ligaments, and made serial sections of six femoral heads, including two in which there had been fractures of the femoral neck. The subjects were adults, averaging 48 years. The ligament was absent in only one case and all others contained vessels. In four, the vessels were of pre-capillary size but in the others they carried a significant blood supply. In six specimens, Nordenson in 1938 suggested that these vessels, even although arteriosclerotic, were capable of hypertrophy, and that this capacity might explain why necrosis of the head was not more common. There was no difference in the blood supply between the males and females particularly at the age of maturation in the 46 specimens of the upper end of the femur that he studied. [27]

Wolcott published an article in 1943 where he investigated the arterial pattern at various ages up to adolescence. He had previously made similar investigations in adults. His conclusions are important:

1) In infants and children, the ossifying centre in the developing head of the femur receives its blood supply from capsular vessels which arise from the medial circumflex artery. 2) The ligamentum teres vessels do not enter the head of the femur in children, nor do they contribute to the nourishment of the growing femoral head, except for very small vessels at the site of implantation of the ligament into the foveolar area. 3) Anastomoses between vessels of the ligamentum teres, capsular arteries, and nutrient arteries of the shaft, do not take place until ossification of the femoral head is almost complete, by which time the vessels of the three systems unite by penetrating the thinned area of cartilage at the fovea. 4) The ligamentum teres circulation is closed, so far as the femoral head is concerned, until such anastomosis takes place.

Retinacular arteries

These vessels arise from the medial and lateral femoral circumflex arteries. There is, however, a brisk extracapsular anastomosis in the region of the trochanteric fossa to which the inferior gluteal, profunda femoris, obturator, and circumflex arteries contribute. The circumflex arteries lie superficial to the distal part of the fibrous capsule and they do not run within its substance. Branches of the arteries pierce the fibrous capsule near its lateral extremity and run medially along the neck of the femur, deep to the reflected cuff of synovial membrane. It is in this position that the vessels are associated with retinacular fibres. As a rule they are found in groups, although occasionally a few isolated and separate vessels may be observed.

There are three main groups of retinacular arteries - the posterosuperior, posteroinferior and anterior. The first two groups are branches of the medial femoral circumflex artery and they run along the upper and lower borders of the neck of the femur. If one looks at the head and neck of the right femur from the medial aspect, the posterosuperior vessels are found between eleven and two o'clock, and the posteroinferior vessels between five and seven o'clock. Although the groups may extend on to the front of the neck, they are usually posterior. These two groups are moderately large and quite consistent, the **postero-superior group being usually the larger, and occasionally providing the**

sole supply to the epiphysis. These are branches of the lateral epiphyseal vessels, which are branches of the distal part of the medial circumflex artery. As the intermediate stage of vascular development of childhood is reached it is characterised by marked individual and racial variations**.** [27]

After age four, **the cross connection between the metaphyseal and epiphyseal arteries begin to decrease.** The points of entry *of* the lateral epiphysial arteries are close together in the outer segment of the head, and slightly posterior. A minute metaphyseal artery still reaches the epiphysis in its medial side, and a number of large arteries in the ligamentum teres end without any connection with the epiphysis.

Some of the infantile metaphyseal vessels may still remain. Apparently, this double supply is an arrangement that protects the nourishment of the epiphysis during the transient intermediate period which extends from four to seven years. More commonly, the distribution of the metaphyseal arteries at about six years of age is like that of the adolescent, when they have finally disappeared, but the infantile absence of blood flow from the ligamentum teres still persists. The picture opposite is a good example of this intermediate vascular pattern. In the pre-adolescent period i.e. the age of seven upwards, the penetration of vessels from the ligamentum teres appears to be increasingly frequent, and at nine or 10, the pre-adolescent type of epiphyseal blood supply seems definitely established, even if sometimes the participation of the medial epiphyseal arteries does not seem as great as in adult life. The growth plate is an isolating barrier raised between the femoral epiphysis and the rest of the bone, and this barrier persists till epiphyseal fusion is complete.

Foveolar artery/ Artery of the ligamentum teres

In children up to the age of 13 years, in eight specimens out of 24, the artery penetrated the fovea and supplied the deep cartilage of the head or the ossific centre. The vessels were very small and varied from one to five in number. In the other 16 specimens, the vessels spread out over the surface of the fovea like the fingers of an outstretched hand, obviously being concerned solely with supply of the fibrous tissue of the ligament and its attachment to the cartilaginous head.

Relative importance of the retinacular and foveolar vessels in children

The cartilaginous head and the ossific centre are supplied almost entirely from retinacular vessels, while in a few specimens the foveolar vessels contribute. In the series reported, foveolar vessels were of importance only in a minority of cases. If this view is accepted, the site of obstructive vascular lesions causing avascular necrosis must be located in the retinacular group of vessels. The foveolar supply may afford additional protection to the ossific centre. The epiphysis in the child is more dependent upon retinacular vessels than is the corresponding area of bone in the adult, thus explaining the greater frequency of avascular necrosis in children

Perthes' disease- If injury is the cause of Perthes' disease, vulnerability of the epiphysis to infarction will be greatest when the blood supply is derived largely from the posterosuperior vessels. In other words, multiple sources of vascular supply provide a safeguard to the nutrition of the femoral epiphysis; in contrast, concentration of the vascular supply to one group of vessels constitutes a potential danger. Tucker believed that the posterosuperior group of vessels was susceptible to pressure from the acetabular lip and its labrum in positions of forced abduction and external rotation of the hip, and that it is individuals with this pattern of vascular supply who were most prone to Perthes' disease. [27] This was confirmed in children with Perthes' disease by Atsumi et al who did super selective angiography in 28 hips (25 children) and noted the disruption of the lateral epiphyseal arteries in 19 of the 28 hips (68%). This decrease in blood flow was more acute in the internally rotated and abducted position. Features of revascularization were obvious by angiography even at stage one disease. [28]

III.iii.c Normal clotting mechanism and basic pathology of thrombosis

The coagulation cascade of secondary hemostasis has two pathways, the Contact Activation pathway (formerly known as the Intrinsic Pathway) and the Tissue Factor pathway (formerly known as the Extrinsic pathway) that lead to fibrin formation. Figure ## It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the Tissue Factor pathway. The pathways are a series of reactions, in which an enzyme and its co-factor are activated to become active components that then catalyze the next reaction in the cascade.

The Virchow's Triad of blood abnormalities, vessel wall abnormalities and flow abnormalities, as causes for thrombosis, still holds good. This theory states that thrombosis in vessels is multifactorial and the three things that influence this are blood composition- any abnormalities causing an increase in thrombotic tendency will fall under this category, the vessel wall – which if damaged can initiate the clotting cascade, and the flow i.e. if there is turbulence there is a higher chance of clot formation.

The causes for an increased risk for thrombosis can be classified under either congenital or acquired causes. Congenital causes include Antithrombin deficiency, Protein C and Protein S deficiencies, The Factor V Leiden mutation, and MTFHR and Prothrombin variants. There is good evidence to show that factor elevations that do cause thrombosis are also clustered in families (such as FVIII) but the mode of inheritance has not been identified. Acquired causes of thrombosis include malignancy, PNH, anti-phospholipid antibody syndrome, smoking, obesity, OCPs, immobilization, pregnancy, dysfibrinogenemia etc.

The most often identified sites for venous thrombosis in the western population were the deep veins of the lower limb and pelvis. The causes for deep vein thrombosis (DVT) in the western population have been investigated and the general profile quite well documented. Taking this as a starting point – i.e. assuming that the same process was at work in the femoral head as well –clotting abnormalities were looked for in Perthes' disease and first reported in the mid nineties. Each of the factors that were implicated will be discussed separately under subheadings.

In India, the incidence of DVT has been recorded in the King Edward Memorial Hospital in Bombay and in C.M.C., Vellore. The rates of DVT measured in Vellore were 7/100,000 and the rates of pulmonary embolism were about 6/100,000. In K.E.M. Hospital, the rates were lower and have been quoted as 3.7/100,000 for DVT. These values are about 10 times lower than in the West where rates of DVT are as high as 48/100,000 per year and the rates of Pulmonary embolism as high as 23/100,0000 as reported by Anderson et al. [29]

In the West, Protein C, Protein S, antithrombin (AT), the factor V Leiden and prothrombin mutations were present in about one third of unselected patients with venous thromboembolism. In addition to these inherited thrombophilic defects, elevated plasma levels of factor VIIIc have been suggested to be important in the pathogenesis of venous (and recurrent) thromboembolism. The Factor

V Leiden mutation was reported to be the commonest abnormality in DVT in the western population with rates of prevalence between five to 30%. The data from our clinical pathology lab show that the incidence of this abnormality is much lower here than in the western population. [30] [31]The incidence of clotting factor abnormalities in Perthes' disease have been reported only recently, the first report being in 1994. These studies showed low levels of protein C, protein S and hypofibrinolysis as causes for clotting abnormalities.,[1,2,3],An elevated level of homocysteine is an independent risk factor for cardiovascular diseases and is associated with other complex disorders. Homocysteine levels can be elevated due to dietary and/or genetic factors. The role of Homosysteine levels in Perthes' has not been examined by most studies. [32], [33], [34], [35]

FACTOR VIII LEVELS

High levels of FVIII were recently determined to be a possible independent major risk factor for deep-vein thrombosis. Whether high FVIII levels after previous thromboembolism are acquired or hereditary is still open to debate. The normal values of Factor VIII is considered to be 1000 IU and concentrations are reported as a percentage; a FVIII concentration of 1000 IU/L corresponds to 100%. Genetic defects that promote high FVIII levels have not been found so far.

One study showed that Factor VIII levels in patients with unexplained thrombosis had relatives with high levels of thrombosis- suggesting that the levels of FVIII are high in some families. [36] Others have demonstrated that a high FVIII level is persistent over time and is a risk factor for thrombosis, independent of the acute phase state. However, it is unknown whether there is a critical cut-off value above which the risk increases or if risk increases continuously with increasing levels of FVIII. Patients with FVIII levels of ≥ 150 IU/dL had an adjusted odds ratio for risk for DVT of 4.8 (95% CI, 2.3 to 10.0) compared with patients with FVIII levels ≤ 100 IU/dL. Hence, high FVIII concentrations represent a risk for thrombosis similar to those of deficiencies of inhibitors like proteins C and S and APC resistance. [37], [38]

A population-based patient-control study was done in which 301 consecutive patients younger than 70 with a first, objectively diagnosed episode of venous thrombosis and without an underlying malignant disorder were compared with 301 healthy controls matched for age and sex. In this study by

Schambeck et al, for individuals with factor VIII concentrations higher than 1500 IU/L, the risk of thrombosis was six times higher than that for subjects in the reference category (<1000 IU/L). This high-risk stratum comprised no less than 25% of the patients and 11% of the controls. The reported associations did not change with adjustment for body-mass index, diabetes mellitus, and smoking status. The prevalence of factor VIII levels above 1500 IU/L was also high among the healthy control subjects (11%). [36]

Koster et al felt that raised factor VIII, similarly to protein C deficiency and activated protein C resistance was not in itself sufficient to cause thrombosis. However, once the other required causal factors were present, high factor VIII concentrations presented a strong thrombosis risk, which was about as high as the relative risk of thrombosis for deficiencies of the major anticoagulants and a poor response to activated protein C.[39]

In a study by Tirado et al, patients with thrombosis showed higher FVIII levels than the controls (200.1± 75.9% versus 151.9± 57.7%, p<0.0001). When FVIII levels were analyzed among different blood groups, significantly higher levels were found in the patients than in the controls. The risk for thrombosis attributed to FVIII levels higher than the 90th percentile was OR 2.9..They also observed that the increase in risk due to FVIII levels was continuous, that is, patients with higher levels had higher risks.[40]

Inherited and acquired thrombophilia are also associated with recurrent pregnancy loss. Dossenbach-Glanninger et al evaluated the relation between recurrent early pregnancy loss and levels of FVIII in patients with multiple pregnancy losses and found that there was a significantly higher level of FVIII in those patients with recurrent pregnancy loss with a relative risk of 3.9 for those patients who had a level of factor > 156% (95th percentile).[41]

In a case-control study performed by Kupferminc et al, [42] about 65% of vascular complications in pregnancy were associated with acquired or genetic thrombophilia. Recently, a metaanalysis of 13 studies confirmed the association of Factor V Leiden mutation i.e. a prothrombotic risk factor with early pregnancy loss. [43] This lends support to the claim that FVIII, similar to other prothrombotic factors, is an independent risk factor for thrombosis. In India, a study done by Shah Sudeep et al in patients with

portal vein thrombosis revealed that about 25% (6/26) of patients had elevated FVIII levels – showing that in the Indian population, Factor VIII may be a significant cause of thrombosis.[44]

There has been debate as to whether the rise in factor VIII levels is a primary elevation or an acute phase reactant that gets elevated in response to the thrombosis per se. The argument that the thrombotic event itself may cause an inflammation and result in the elevation of factor VIII levels and fibrinogen has been disproved by the study by Kraaijenhagen et al. [45] They measured the levels of CRP(an acute phase reactant marker) and fibrinogen and factor VIII and showed that there was no significant association between these. This study has supported the reasoning that elevation of FVIII is independent of an acute phase reaction. They found that an elevated factor VIII plasma concentration was a prevalent, independent and strong risk factor for venous thromboembolism. Increased factor VIII levels (above >175 IU/dl) may be found in approximately one quarter of unselected patients with symptomatic venous thromboembolism, which is in agreement with earlier observations.

For each 10 IU/dl increase of factor VIIIc, the risk for venous thromboembolism in all patients increased by approximately 17% and in recurrent thromboembolism by 24%.[46] O'Donell et al followed up a cohort of patients with elevated FVIII levels who had DVT and found that 94% continued to have an elevated FVIII:C level throughout the period of follow up (median 8 months; range 3 to 39 months), with no significant difference between the FVIII:C levels determined at first estimation and those obtained during follow up ($p = 0.58$). Only 18% of these patients had an elevation of an acute phase reactant - again supporting the hypothesis that the level of FVIII is not an acute phase reactant. [30],

Protein C

Protein C is a circulating glycoprotein that is a part of the negative feedback system of blood clotting. A low level of Protein C increases the tendency to clot i.e. thrombophilia. The levels of protein C are known to be lower in children than in adults. [47]

This deficiency of protein C can be acquired or inherited. The population frequency of Protein C levels in India has not been reported. Though the deficiency of Protein C for the Indian population with thrombophilic events has not been reported, data from our institution shows an incidence of 9% in those with DVT (2005 – 2006 unpublished data of 606 patients).

Protein C circulates in an inactive proenzyme form normally and is activated by thrombin to become functional as an anticoagulant. Once activated, it is known as activated protein C (APC) and begins to deactivate factors V and VIII and also stimulates fibrinolysis as well as preventing formation of thrombin.

Resistance to APC (APCR) in an activated PTT assay is a recently described mechanism for thrombophilia. (1). The most well-known of the causes for APCR is the Factor V Leiden Mutation that is characterised by the substitution of the arginine residue at 506 to glutamine. [48,49,50,51,52]

The incidence of familial Protein C and protein S deficiencies are rare in the western population with a heterozygote prevalence of one in 16,000 to 20,000 to one in 200 to 300.[53] In patients with thrombophlebitis the incidence is less than 5%. As the incidence of Perthes' disease in India varies from 0.4 per 100,000 to 4.4 per 100,000 in Vellore per year as reported by Joseph et al . [17], by chance alone the estimated likelihood of a child having LCPD and a concurrent deficiency of Protein C would be less than 1 in five hundred.

In 1994, Glueck et al [3] published their first study showing five of eight patients with a thrombophilic tendency of whom three had a protein C deficiency. Glueck et al studied 44 unselected children with Perthes' disease of which 33 (75 %) had coagulation abnormalities and 19 of these 33 had a protein C deficiency. In 11 of the 19 children who had a low protein C level, at least one of the first-degree relatives of had a low protein-C level as well; all of these low levels represented previously undiagnosed familial protein C deficiency. These 11 children who had familial protein C deficiency were more likely to have early onset of Legg-Perthes' disease (at or before the age of 5) than the eleven children who were completely normal (chi-square = 6.6; p = 0.01). [1].

Manucci and Vigano have reported that the levels of protein C may be reduced by either infection or injury-[53]. This will definitely influence the levels of protein C measured as most of the studies on clotting abnormalities in Perthes' disease were done on a retrospective basis and most, long after the onset of Perthes' disease. Transiently low levels of Protein C secondary to an infection will therefore be missed.

Eldridge et al conducted a case-control study among 57 patients with Legg-Perthes' disease and measured protein C and protein S and resistance to activated protein C (APC-R) from plasma.

They observed a statistically significant increased risk of Legg-Perthes' disease with decreasing levels of protein C. The factor V gene (contributing to APCR) defect was present in five (9%) of 55 cases and three (5%) of 56 controls (odds ratio 1.8, 95% confidence interval: 0.4 –7.7), but the mean level on the APC-R plasma test was similar for cases and controls. Nine cases and one control had two low normal or low test results (odds ratio 13.0, 95% confidence interval: 2.2–75). Their results support the belief that abnormalities of the coagulation system leading to a thrombophilic state play a role in Legg-Perthes' disease.[54] This was a retrospective study and the same problems with respect to testing of values sometimes long after the onset and resolution of the disease hold good here as well.

Protein S

Glueck et al studied 44 unselected children with Perthes' disease of whom he found of which four had protein-S deficiency. At least one first-degree relative of one of the four children who had a low protein-S level, had a low protein-S level and previously undiagnosed familial protein S deficiency1

Eldridge et al conducted a case-control study among 57 patients with Legg-Perthes' disease observed a nearly significant increased risk with decreasing levels of protein S. Their results lend support to the belief that abnormalities of the coagulation system leading to a thrombophilic state play a role in Legg-Perthes' disease. [54]

Factor V Leiden mutation and Activated protein C resistance

In their study of 44 children with Perthes' disease, Glueck et al reported that three children had hypofibrinolysis (a reduced ability to lyse clots) and seven children had a high level (0.25 gram per litre or more) of lipoprotein(a), a thrombogenic, atherogenic lipoprotein associated with osteonecrosis in adults. [1]

Glueck et al in 1997 investigated 64 patients with Perthes' disease in whom they examined the factor V Leiden mutation and found that 8 out of 64 children had the mutant Factor V gene as opposed to one of 101 normal controls. There was evidence of familial involvement with this trait.[55,]

In the 47 patients with Perthes' disease included in the study by Szepesek et al, 10.6% had the Factor V Leiden mutation and of these 4 were homozygous for FVL. All these patients had Caterall IV

disease and head at risk signs. This is suggestive that severe disease is associated with thrombotic tendencies. [10,]

Balasa et al in 2004 did a prospective study on 72 children with 197 controls and found that the FVL mutation was more common in the patients (8 of 72) that in the controls (7/197). After making adjustments for a false discovery rate they found a significant difference between the groups. The odds ratio for developing Perthes' disease was 3.39. They felt that the FVL mutation was associated with Perthes' disease and this may reflect causality. [2]

Arruda et al carried out an investigation of FVL, the transition 20210 G->A in the prothrombin gene, and also the homozygosity for the 677C->T transition in the methylenetetrahydrofolate reductase gene (MTHFR). The investigation was carried out among 61 Brazilian children with LCPD, who were compared with 296 individuals from the general population. The prevalence of the factor V Leiden mutation was higher in LCPD patients than in the controls (4.9 vs. 0.7%; p = 0.03). However, no patient had the prothrombin gene variant, and no difference was found between patients and controls when homozygosity for MTHFR-T (3.2 vs. 2.6%; p = 0.64) was determined. They felt that the heterozygosity for factor V Leiden was the only inherited risk factor associated with the development of LCPD in this subgroup of Brazilian children. [56.]

ANTIPHOSPHOLIPID ANTIBODY

Anticardiolipin antibodies, lupus anticoagulant, and antibodies causing false positive tests for syphilis belong to a family of autoantibodies called antiphospholipid antibodies, which are active against negatively charged phospholipids and are clinically manifested by an increased tendency for thromboembolic events. Although it is well established that their presence is associated with thrombocytopenia, midpregnancy recurrent fetal loss, stroke, and cardiac valvular disease, the main associated feature is venous and arterial thrombosis. Vessels of all sizes may be involved, including the aortic arch, pulmonary and cerebral vessels, coronary arteries, retinal arteries, peripheral arteries, and smaller skin vessels. The combination of recurrent thrombosis and antiphospholipid antibodies in patients without features of systemic lupus erythematosus is called primary antiphospholipid syndrome. The syndrome is secondary if the patient also has systemic lupus erythematosus or a lupuslike disease.

Balasa et al in 2004 did a prospective study on 72 children with 197 controls and found that 19 of 72 patients as compared with 22 of 197 patients had elevated anticardiolipin antibodies. After controlling for the false discovery rate, they found that there was a significant difference between the two groups. [2]

Korompilias et al studied the prevalence of anticardiolipin antibodies, in 40 consecutive patients (25 men and 15 women) with nontraumatic osteonecrosis of the hip, which have been associated with thrombotic phenomena. Their ages ranged from 19 to 56 years (average, 34.3 years). Anticardiolipin antibodies were present in 37.5% (15 of 40) of the tested patients, a significantly higher rate than is seen in healthy subjects, of whom only one of 100 had low titer anticardiolipin antibodies (1%). Six of 40 patients tested positive for immunoglobulin M alone, and six of 40 patients tested positive for immunoglobulin A alone. Three of 40 patients tested positive for immunoglobulin M and immunoglobulin A isotype. They reported that the results of their study indicated an increased incidence of anticardiolipin antibodies in patients with nontraumatic osteonecrosis of the femoral head, probably reflecting that anticardiolipin antibodies play a role in the pathogenesis of bone necrosis by predisposing to thrombotic phenomena. [57] Chan et al have reported that APLA is associated with VTE in children with systemic lupus erythematosus.[50,]

Homocysteine

Homocysteine, a sulphur-containing amino acid, is an intermediate formed during the catabolism of the essential dietary amino acid methionine as a by-product of biological methylation reactions. Approximately 80% of plasma homocysteine is protein bound. Only a small amount exists as free reduced homocysteine, as the majority of the unbound portion is oxidized to form diamers (homocystine) or combined with cysteine to form mixed disulphides. Hyperhomocysteinemia (HHcy) is an independent risk factor for cardiovascular disease, including ischemic heart disease, stroke, and peripheral vascular disease. Mutations in the enzymes responsible for homocysteine metabolism, particularly cystathionine b-synthase (CBS) or 5,10-Methylenetetrahydrofolate reductase (MTHFR), result in severe forms of HHcy. The reaction with MTHF occurs in all tissues and is vitamin B12–

dependent, whereas the reaction with betaine is confined mainly to the liver and is vitamin B12–independent.

Additionally, nutritional deficiencies of vitamin B cofactors required for homocysteine metabolism, including folic acid, vitamin B6 (pyridoxal phosphate), and/or B12 (methylcobalamin), can induce HHcy. Studies using animal models of genetic and diet-induced HHcy have recently demonstrated a causal relationship between HHcy, endothelial dysfunction, and accelerated atherosclerosis. Dietary enrichment in B vitamins attenuates these adverse effects ofHHcy. [35]

The study by Jakubowski supports a hypothesis that protein *N*-homocysteinylation and resulting protein damage cause homocysteine toxicity to human cells, especially to vascular endothelium. The homocysteine theory of atherosclerosis arose from the observation that diseases such as homozygous homocystinuria, which are characterized by severe hyperhomocysteinaemia (>0·1 mmol . l_1), are associated with premature vascular disease.

Jakubowski suggests that homocysteine is atherogenic in places where arteries branch out as these branches have a high local concentration of NO which precipitated the incorporation of homocysteine into proteins and causes vascular endothelial damage. This is accentuated in patients where the levels of homocysteine are high due to different reasons. [32]

The epidemiological evidence for the homocysteine theory of atherosclerosis is based largely upon pathophysiological homocysteine concentrations of 0·01– 0·03 mmol . Patients with homozygous homocysteinuria have plasma concentrations of upto 0·4 mmol . However, many of the in vitro studies described above demonstrated adverse effects of homocysteine at concentrations of 1–10 mmol. making the pathophysiological significance of these experiments difficult to interpret. Despite these criticisms, there are several plausible mechanisms for homocysteine-induced atherogenesis and thrombosis: such as 1. Endothelial cellular and DNA damage 2. Reduced activity of glutathione peroxidase 3. Stimulation of procoagulant and impairment of anticoagulant and fibrinolytic pathways 4. Mitogenic effect on smooth muscle proliferation 5. Promotion of endothelial–leukocyte interactions. [34]

Although venous thromboembolism accounts for 50% of the vascular complications of homocystinuria, the link between less severe hyperhomocysteinemia and venous thromboembolic disease was overlooked until recently. Recurrent episodes of thromboembolism, events that occur at an

early age, thrombosis after trivial provocation, and thrombosis at unusual sites are all features which should heighten the suspicion that an inherited metabolic abnormality is playing an etiologic role. Most studies done on thrombophilia and Perthes' disease have not examined the relation between the disease and the MTFHR gene which results in elevated homocysteine levels. [35] Only Arruda et al have examined the incidence of the MTFHR gene and did not find any significant association with the at risk population. [56]

ANTITHROMBIN III

Rosendaal mentions that the first report of a family with an identified hereditary tendency to thrombosis (a deficiency of antithrombin, previously known as antithrombin III) was made by Egeberg in 1965. Since then, it has been described as an independent risk factor for thrombosis and therefore was examined in our study as a part of the work up for thrombosis.[58]

Fibrinogen

Koster et al from the LETS study group reported a positive, level-related, association between plasma fibrinogen level (as measured with the Claus assay) and risk of venous thrombosis. This conclusion was based on the analysis of the first 199 patients and 199 controls of the Leiden thrombophilia study (LETS). Later the analysis was extended to all the 474 patients and controls of the LETS. Subjects with plasma fibrinogen above the 95th percentile of the distribution measured in the control subjects, had a 2.8 fold increased risk of a first deep vein thrombosis. This risk was found to be equal in men and women, and even higher (OR 4.2) in subjects older than 45 years. Adjustment for other risk factors (prothrombin, FVIII, FIX, FXI, FV Leiden, PT20210A) and C-reactive protein reduced the OR to 1.5 (95% CI of 0.8- 3.0). This finding indicates that the effect of elevated fibrinogen levels on thrombotic risk might be very small, even in subjects older than 45 years. [59]

MULTICAUSAL DISEASE

Thrombosis manifests itself as a multicausal disease most clearly in children. In the rare event of thrombosis in children, several acquired and genetic risk factors are usually present simultaneously.

Not only is it rare to find children with thrombosis without any risk factor, but many have three or four risk factors. In 25–30% of children with thrombosis, deficiencies of protein C, protein S, or antithrombin have been reported, but thrombosis did not develop until other risk factors were present, such as intravenous lines or major illness.[50] Thrombosis is also a multicausal disease in adults since many risk factors are common in the general population, such as factor V Leiden, prothrombin 20210A, high concentrations of factor VIII, and hyperhomocysteinaemia, which frequently occur together in one individual. The acquired risk factors, such as pregnancy, puerperium, use of oral contraceptives, and immobilization also affect many people, and so a combination of risk factors in one person is common. Indeed, multiple risk factors often are a prerequisite for thrombosis to develop. Since some of the recently discovered genetic abnormalities are common, as are several acquired risk factors, the joint effects of such factors on the risk of thrombosis warrants investigation. Clear indications of synergistic effects come from studies in thrombophilic families, where high risks were found in pregnancy and puerperium, and during use of oral contraceptives for women with deficiencies of protein C, protein S, or antithrombin. In several series of unselected women with thombosis during pregnancy, factor V Leiden was more common than in the general population. The frequency of factor V Leiden among these women varied widely between studies, from 8% in Scotland to 50–60% in Sweden, which partly reflects geographical differences in the population prevalence of factor V Leiden. These data suggest that a substantial part of pregnancy-related thrombosis results from concomitant abnormalities in the haemostatic system. Among unselected patients, a synergistic effect has been shown for factor V Leiden and use of oral contraceptives: the estimated baseline risk of thrombosis for non-carriers who do not use oral contraceptives was 0·8 per 10,000 people per year. The annual risk for women with factor V Leiden who did not use oral contraceptives was 5·7 per 10,000 people (relative risk 6·9), that for women who used oral contraceptives but did not carry factor V Leiden was 3·0 per 10 000 women (relative risk 3·7), and that for women with factor V Leiden who used oral contraceptives was 28·5 per 10 000 people (relative risk 34·7). For cerebral sinus thrombosis, increased risk of thrombosis has been reported for thrombophilic defects. [60]

III.iii.b Vascular theory – interruption of femoral arterial supply or venous drainage:

In the hip joint, there are two unusual features that place the joint at risk when subjected to injury, namely, the fact that the capsule is virtually incapable of being distended and because, as discussed in the section on anatomy, due to the unique blood supply to the hip joint, the vessels can also be affected.

Kemp, in 1981, did an experimental study on dogs where he suggested that the degree of epiphyseal involvement may be related to the intensity of the intracapsular tamponade. The reason why he chose dogs to do this study was that there is a close parallel between the developing blood supply in the child and in the puppy and that there is a progressive incorporation of the blood vessels into fibro-osseous canals in the femoral neck as maturation takes place. In some breeds of puppies resistant to Perthes' disease, the venous tamponade was present only with an intracapsular pressure in excess of 120 mm Hg (the systolic pressure of the experimental animal). In contrast to the response of the resistant animals, the marrow pressure in the susceptible poodles showed an immediate response to tamponade, responding sensitively to the progressive elevation of intracapsular pressure and this was thought to be due to the fact that the draining veins were intracapsular. He felt that this evidence obtained from experiments on dogs suggested that transient venous occlusion could produce coxa plana. [61]

In 1986, Kemp performed intracapsular tamponade of the hip joint under anesthesia in rabbits and puppies by the injection of wax that was liquid at body temperature to simulate an effusion. The hips of these animals when examined microscopically showed features consistent with Perthes' disease and this study supported venous occlusion as a possible etiology for Perthes' disease. He felt that if the disease was to occur in a particular hip, then two factors were required- first, an intrinsic factor, which he felt was the delayed skeletal growth and the second, an effusion in a susceptible joint. He felt that in a susceptible hip joint, an effusion could produce an obstruction of the venous return, because the hip capsule is incapable of distension and this could make the epiphysis (and possibly the associated metaphysis) vulnerable to transient anoxia. [62] Chung demonstrated that the anterior vessels had much less anastomoses than the posterior vessels and these anastomoses were more often incomplete in boys and attributed the higher incidence in boys to this phenomenon. There was also an attenuation of

the extracapsular network in children aged 3- 10 years – a normal process that occurs during growth. This may be why Perthes' disease occurs more often in this age group. [63] Most of the work done on the blood supply of the hip, both in children and in adults, was influenced by. Prof Joseph Trueta.[64, 65.] Deficient venous drainage as the cause for vascular insult was suggested by Heikkenen et al. They proved that poor venous drainage was a prognosticator for severity of disease. [66.] An animal study in by Liu and Ho revealed a higher venous pressure in femoral heads of children who were affected by Perthes' and suggested that this venous hypertension may be the precipitating factor for thrombosis. They then did a follow up with a study on dogs and were able to demonstrate this experimentally. [67]

Harrison and Burwell, in 1981 wrote that the concept of extraepiphyseal vascular interference was attractive, but was inconsistent with clinical facts such as the fact that the vascular vulnerability of the epiphysis was limited to the interval between the third and eighth years. They felt that the tamponade ought to be painful due to the avascualrity but the onset of Perthes' disease is usually not marked with an episode of acute pain and hip spasm. Bilateral Perthes' disease occurred in over 10% of patients, the disease affected four boys to one girl and not rarely more than one child of a family; in a number of families, more than one generation were affected. Though the former points still hold good, the rest can be reevaluated in the light of the fact that thrombosis could cause occlusion in the susceptible hip and that it was not yet discovered then. However, they also said that Perthes' disease could no longer be considered a hip disease in an otherwise normal child, but rather a focal expression of a general disorder of skeletal growth. A hypothesis based on these observations is that Perthes' disease is a force-oriented hip lesion based upon a constitutional defect which affects growing bones. The infarction in Perthes' disease was self-perpetuating and healing was proportionately slow. [68]

Sanchis et al proposed the double infarction theory where they experimentally infarcted the femoral capital epiphyses of puppies. They were unable to demonstrate the typical histological picture of Perthes' disease with only a single infarction. After repeating the infarction again they were able to show a more characteristic picture of Perthes' disease. [69] Clinical correlation for this theory is provided by reports of recurrent Perthes' disease in children by Katz as well as Martinez and Weinstein.[70,71.]

That the vascularity of the femoral epiphysis was embarrassed is beyond question; whether it was arterial or venous remained.

III.ii.c Perthes' disease as a systemic disorder

There are suggestions by authors that Perthes' disease should be called Perthes' syndrome as there are often multiple sites that show the same histological and radiological features of avascular insults. Smith and Nevelos in 1980, described osteochondritis occurring at multiple sites in Perthes' disease that was especially prominent in the navicular. [72.] Bilateral hip disease was reported to be about 11.3% by Wynne-Davies and Gormley in 310 patients. They felt that it was unlikely that there were any genetic factors in the great majority of cases. Bilateral hip involvement was noted in only 6% of patients by Chacko et al, which is less than that reported elsewhere. [24] Pillai et al, in 2005, reported that in 40 children with Perthes' disease from South West Scotland, there was bilateral involvement of the hip in 7.7%. [19]

Harrison and Blakemore reported 48% of contralateral hips having contour abnormalities with only 10% of the control series having the same abnormality (irregularities of the surface and flattening or dimpling of the femoral head. [73.]

Kitoh et al studied radiographs of 125 children (105 boys, 20 girls) with unilateral Legg-Calvé-Perthes' disease to examine the epiphyseal development of the femoral head in the contralateral (unaffected) hip. The epiphyseal height (EH) and width (EW) of the unaffected hip were measured on the initial anteroposterior pelvic radiograph. In 87.2%, the EH was below the normal for children in the same age group. This indicates that there may be abnormal development of the femoral head in this disease. [74.]

This is further evidence of the fact that Perthes' disease is a systemic disease. Burwell et al felt that the skeletal abnormalities they found in their patients could be explained by one of two factors - possible local disorder of allometric growth at the hip and a general disorder of skeletal maturation and felt that these two factors acting together might produce Perthes' disease. [75.]

Endocrine abnormalities

Some aspects of endocrine function have been evaluated in affected children. According to the study by Burwell et al hypothyroidism, growth hormone, follicle stimulating hormone, luteinising hormone and 17- ketosteroid abnormalities were not significantly associated with Perthes' disease. They have mentioned though that some patients with severe growth limitation and Perthes' disease were investigated and about 25% of these children were found to have hormonal abnormalities. They found that in their population there was a tendency to low levels of growth hormone and raised levels of somatomedin in some children with Perthes' disease. [75] Rayner et al noting the frequent association of short stature and retardation of skeletal maturation in Perthes' disease studied a group of 18 prepubertal boys, aged five to 11 years, who had features of growth retardation. Their serum growth hormone (Se GH) response to insulin-induced hypoglycemia was significantly reduced, compared with a control group of short boys. They also demonstrated a tendency to elevated levels of somatomedin activity, measured by chick cartilage bioassay. Thyroid function was normal. They attributed these findings to a probable defect in the pituitary-somatomedin-target tissue axis in Perthes' disease. [76.]

Burwell et al in 1986 examined the Somatomedin C (IGF1) axis in 67 boys with Perthes' disease and 43 control boys aged three to 11 years using a bioassay based on the principle that somatomedins stimulate the synthesis of both DNA and proteoglycans in porcine costal cartilage. In control boys, the serum somatomedin activity increased with age, which is consistent with previous reports for normal children. In affected boys, the normal increase in serum somatomedin activity with age did not occur. The children with Perthes' disease had higher levels of somatomedin activity between three to five years but not at six to 11 years of age. These findings support the hypothesis that some children with Perthes' disease have an abnormality of the growth hormone-dependent somatomedins. The serum findings together with those of both skeletal age delay and impaired skeletal growth distally in the limbs are consistent with the view that the general disorder of some children with Perthes' disease results from an imbalance in pituitary axis. [77.]

Perthes' disease and genetic inheritance

As mentioned in the history, familial history has been noted and the cause for this has been debated for nearly a century now. That Perthes' disease had a familial tendency was reemphasized since the 1980's and has been given mention by Hall DJ, O'Sullivan et al and Wynne-Davies. [78, 79, 80] However, the genetic basis of this was not investigated then and no cause was found for the familial incidence.

Pillai et al in 2005 reported that in 40 children with Perthes' disease from South West Scotland there were three patients who had either a parent or a sibling affected with Perthes'. [19]

The frequency of Perthes' disease among near relatives was extremely low and it rarely involved both identical twins. The proportions of affected second and third-degree relatives dropped immediately to the probable population frequency (about 3 per 1000 males). The reason why males are more commonly affected is still not known- it would be expected that girls would have a higher incidence than noted of Perthes' disease if it was a genetic abnormality. [25]

Hall DJ et al reported on the family data from a series of 87 boys and 58 girls with Perthes' disease combined with those of another study by Gray et al *(*223 boys and 44 girls). Proportions of first, second, and third degree relatives affected were recorded separately for each sex of index cases and each sex of relatives. Comparison of the incidence of Perthes' disease in relatives with that in the general population of the same sex revealed features of multifactorial inheritance, and a gradient of 35:4.4:1 from first:second:third degree relatives to the general population. They argued that the concentration of cases in certain families was not inconsistent with multifactorial inheritance. [79.]

It is not always possible to know whether bilateral Perthes' disease with a strong family history that was reported in previous studies was merely one of the many skeletal dysplasias involving the hip joints i.e. multiple epiphyseal dysplasia or spondylo-epiphysial dysplasias.

Glueck et al have reported a positive family history in their study and also reported incidence of adult AVN and thrombotic episodes as being more common in families of patients affected with Perthes' disease. [1]

Socio-Economic status

Environmental factors were thought to play a part in the aetiology and were investigated.

There are studies showing that there is an increased incidence of Perthes' disease in the urban population in Liverpool, as well as, an association with a lower socio-economic status and since the urban population is from the inner cities, this could be linked. In the study by Barker and Hall, there was a distinct increase of incidence in the inner city wards (the slums) with - 4 per 100,000 in Social Class I to 26 per 100,000 in Social Class 5. [21] A highly significant excess of patients from low income group families (social classes IV and V) was found in the study done by Wynne-Davies and Gormley in Edinburgh and Glasgow City. The "expected" proportion of low income families in this population was approximately 28 per cent, whereas the "observed" proportion among the Perthes' families was 43 per cent. A high significant proportion of these children came from low-income families [25] (43% as opposed to 28% in the normal population).

It was found that the incidence was high in children who were born late in the family. The siblings who were born later were more likely to have had the disease. The age of the parents of involved children was greater than average as compared to the controls in the study by Wynn-Davies and Gormley. [25]

Trauma and transient synovitis

The terms irritable hip, transitory hip arthritis, ' observation hip', transitory coxitis, coxitis serosa seu simplex, acute transient epiphysitis, toxic synovitis and transient synovitis have all been used by various authors to describe this common and fleeting disease of childhood. At initial presentation, transient synovitis and Perthes' disease may be indistinguishable. The argument supporting the theory that transient synovitis causes Perthes' disease is that the intracapsular pressure that is caused by the synovitis often exceeds the venous pressure and this may cause a venous tamponade resulting in avascular necrosis of the epiphysis.

The key feature is that there is a synovitis that often gets better in 10 – 14 days and most by a few weeks. As quoted by Valderrama, in his review of the "observation hip" from the Nuffield orthopaedic centre, Edwards in 1952 thought this to be a "reaction to infection' or "allergic

hypersensitivity" to and infection elsewhere in the body but sometimes the condition followed an injury. From a total of 189 children an arbitrary follow up of 30 years was decided and 23 children with a 30 year follow up were examined- three had bilateral hip involvement at different periods. They suggested that the " observation hip " syndrome is the result of an inflammatory process of the joint due to varied etiology, most often from either injury or infection. They noted developmental and degenerative changes which they felt were a consequence of hypervascularisation of the bone secondary to the synovitis. These changes may develop without necessarily producing the epiphysial necrosis. characteristic of the first stage of ischaemia in Legg-Calvé-Perthes' disease. The persistence of this stage of hypervascularity, and therefore the possible outcome of the transient synovitis, may be conditioned by the age at onset of the pathologcal process, the severity of the condition, and the duration of the symptoms and signs. [81]

Adams in his study of 50 children from South Africa noted that the children with transient synovitis and those who were initially diagnosed to have Legg-Perthes' disease had much the same age distribution but the length of the history was considerably longer in Perthes' disease. Ten percent of the patients with Legg-Perthes' disease had normal or doubtful radiographs at the outset, and in these the history was shorter and the onset acute. Radiographic changes always developed as the condition progressed. Injuries were recorded in a higher percentage of children with Legg-Perthes' disease (19 per cent) than those with transient synovitis (12 per cent). [82.]

Perthes' disease can follow a transient synovitis, but not all cases are so affected. It is reported to occur in up to 12 % of children who had had transient synovitis of the hip by Wynne Davies and Gormley. [25]

Landin et al, in a prospective five-year study from Malmo, found 294 episodes of acute transient synovitis of the hip in *275* children. The risk of recurrence was 20 times greater than the risk of having a single episode. Perthes' disease was diagnosed from one to five months after the acute attack of synovitis in 10 cases (3.4%)**.** Review of the initial radiographs revealed signs of avascular necrosis in three of the 10 cases, and an increased joint space in five. Only two cases had completely normal radiographs. The value of routine radiographs taken after three months was minimal. Factors associated with the incidence of Perthes' disease included prolonged time in traction before the range

of hip movement became normal, increase in joint space on the initial radiographs and the recurrence of hip symptoms after initial relief. [83.]

Pillai et al in 2005 reported that in 40 children with Perthes' disease from South West Scotland history of antecedent trauma was noted in 12.5%. [19]

Kallio et al in Helsinki studied 119 children prospectively with transient synovitis or any other cause for synovial effusion and elevated intra-articular pressure. During a follow-up of one year not one case of Perthes' disease was diagnosed and the late clinical and radiographic changes were minimal with moderate overgrowth of the femoral head in 33% and widening of the joint space in 14.2%. They felt that their results did not support the concept that Perthes' disease develops as a result of the period of elevated intra-articular pressure found in transient synovitis.[84.]

They had two patients with transient synovitis in one hip and symptom-free Perthes' disease in the other hip which they thought was coincidental. Three of the 18 patients excluded with Perthes' disease had been initially diagnosed as having transient synovitis, but in all three retrospective analysis of the original radiographs showed minor changes, such as a slightly widened joint space with a subcortical clear zone after which they were classified as not having synovitis but early Perthes' disease instead. The reason why this was done was not clearly explained. They felt that Perthes' disease may be misdiagnosed as transient synovitis because of scanty or absent radiographic changes and radiographic progression may then be a misinterpretation as development of Perthes' disease after transient synovitis. They also felt that the treatment of transient synovitis by traction with the hip in extension had caused a high intraarticular pressure causing Perthes' disease in some patients.

The serum immunoglobulin levels in Perthes' disease was examined by Joseph et al and he found that there were significant increases in IgG and IgM were seen In Indian children with Perthes' disease in both boys and girls as compared to age and sex matched controls. Matsoukas' study showed an increase in increase in IgG in Perthes' disease, whereas in the former study both IgG and 1gM levels were raised. [85, 86,].

Congenital factors

10.7% (32 / 310) of children investigated by Wynne-Davies and Gormley had a breech birth or other malposition or had had a version late in pregnancy as opposed to 2 - 4% in the normal population. This could have been the first in a series of minor traumatic episodes in some additional cases. [25]

Catterall, Lloyd-Roberts and Wynne-Davies reported an increased frequency of congenital abnormalities of the genito-urinary tract, both in patients with Perthes' disease and in their near relatives, and noted that the chondrogenic cells associated with the mesonephros eventually contributed to the formation of limb girdle cartilage. Thus, in some instances of the disease there could be a primary deficiency either in these cells or in their blood supply. [87,] Wynne-Davies and Gormley reported that 10 male patients had inguinal hernias and there were 4 instances of congenital defects of the kidney, the ureter or hypospadias in 310 patients with Perthes' disease. [25] There was definite family occurrence of renal tract abnormalities in the families of the patients indicating a probable inheritable congenital renal tract defect.

In the spine, Katz found a high incidence of spina bifida occulta (82 per cent) in 178 children with Perthes' disease. Examination of a control series of eighty-nine patients with "all varieties of orthopaedic disorders other than Legg-Calvé-Perthes' disease", showed the incidence of spina bifida occulta to be 67 per cent. Katz concluded that there was no evidence to prove a relationship between spina bifida occulta and Perthes' disease. [88.]

It has been established that some congenital abnormalities show a social class gradient, being more frequent in the children of unskilled workers. Hall, Harrison, and Burwell, in their consecutive series of ninety boys and forty girls from the Birmingham area found a remarkable similarity of the incidence of minor anomalies in children with Perthes' disease to that in babies with a single major congenital defect. This invites the speculation that there is a major congenital defect in these children which in some way makes the hip susceptible to Perthes' disease at a later date. [89.]

Loder et al have linked Perthes' disease with behavioral profiles and found this to be associated with attention deficit hyperactivity disorder. [90.]

MATERIAL AND METHODS

The power, too, to study correctly what has been written, I consider to be an important part of the *art* of medicine.

Hippocrates

IV Material and Methods

IV.i. Study design

This study was a prospective case control study that was based on the diagnosis of Perthes' disease in the setting of the outpatient department of our Paediatric Orthopaedic clinic. We assumed the prevalence of disease for our study to be 0.4/100,000. [17] There are no population based studies for the exposure factor- i.e. thrombotic factors. The data from our clinical pathology lab suggests that the incidence of DVT in our population is around 7/100,000. The risk of having thrombotic tendencies in a subpopulation with DVT is 60% (Unpublished data 2005-6, Christian Medical College, Vellore).Thus extrapolating from this the average prevalence of the exposure factor is 4.2/100,000.The prevalence of thrombotic tendencies among the subpopulation with Perthes' disease varies with several studies- from 75% to 12%. Using the Epiinfo statistical package we calculated a sample size of 17 cases for 95% CI and 80% power using the same number of controls.

IV.ii Definitions

1. Perthes' disease was diagnosed based on both radiological and clinical features. Clinical features were hip pain, referred pain to the knee, limp and limitation of movement in association with radiological features of avascular necrosis of the capital femoral epiphysis.

IV.iii Sample selection

1. Cases: Children with Perthes' disease.

    a. Severe disease: Classified as either Salter Thompson B, Caterall group III or IV, or Herring's Type C if the patient presented to us with collapse. As the subchondral fracture line was not visible in some cases it was not possible to classify each individual case into the Salter Thompson classification. The stage of the disease was classified using the modified Elizabethtown classification as reported by Joseph et al. [91, 92, 93,100.]
    b. Familial Perthes' disease – if at least two siblings had Perthes' disease.

c. Recurrent Perthes' disease.

2. Exclusion criteria

    a. If the patient had chronic infection as this is known to raise the clotting factor levels as a part of the acute phase reaction.
    b. If the patient had surgery within six months of presenting to us which may raise the levels of clotting factor levels as a part of the acute phase reaction.
    c. Children with 'mild' Perthes' disease were not included in the work up for thrombosis.

IV.iv Radiological classification

This was done as described earlier. The Caterall classification gauging the extent of femoral head involvement was used and group III and IV disease was classified as severe involvement. Herring's classification was used to assess the lateral pillar and Herring C was classified as severe disease. The Salter-Thompson classification which uses the extent of the subchondral fracture line was also used and all type B were classified as severe disease. We used the modified Elizabeth town staging as suggested by Joseph et al. [91,]

IV.v Thrombophilia Testing

    d. The cost of doing the thrombophilic work up was Rs.9300/-, we decided to include only the children with severe disease. Since the tests were expensive, the levels of the factors in cases were first analysed to see if they fit into the internationally accepted standards for normal factor levels. If there were any values above the cutoffs for the normal values, the tests were run on the controls as well. Only the fibrinogen and protein S free levels were found to be totally within the normal range and so control values were not done for them. All the other factors had values close to the cut off values for normal and controls were done for these as we wanted to establish if there was a relationship between the absolute values of the tests and the tendency to develop Perthes' disease.

e. Blood collection: All cases had their blood collected after a 12 hour fast in the morning between 8.00 and 9.00 am to reduce the effect of circadian rhythms on the levels of factors. The samples were drawn for both the DNA based tests and the thrombotic work up at the same time. Informed consent was taken from the parents at the out patient department.

f. Samples: the samples were centrifuged at 3000 for 40 minutes and snap frozen at -86° Centigrade for testing at a later date. The DNA Samples were processed the same day in the Department of Haematology.

g. The machine used for analysis and the reagents for the prothrombin and Partial thromboplastin time was from the Instrumentation Laboratory (Milan, Italy). The reagents and analysis of Fibrinogen, Factor VIII, and APCR was also from the Instrumentation laboratories. The reagents for DRVVT was from Gradipore(Life Diagnostics, French's Forest, NSW, Australia). The reagents for Protein C and Antithrombin were from Dade-Behring, Marburg, Germany. Protein S was evaluated using an ELISA kit sourced from Dako (Denmark).The free Protein S was done using reagents from Coregenic, Westmin, Germany. Sickle cell and paroxysmal nocturnal haemoglobinuria was evaluated at the special test labs.

3. We also collected data on the age, sex, address, anthropometry(height and weight), family history of disease, trauma and fever, as well as coexisting disease.

4. Statistical analysis

a. The Fisher's exact test was used for categorical variables and the students t-test for normal and Mann-Whitney U test for the non normal continuous variables. All values are expressed as the mean ± Standard Dev (continuous variables) or as a percentage of the group they were derived from(categorical variables). All p values of ≤ 0.05 were considered to indicate statistical significance. This was calculated using the software package SPSS Version 9 for Windows.

Results

Number of cases and number of controls

There were 20 cases and 23 controls. The radiographs and other data were analysed for 20 cases.

Age

The mean age of presentation of children with Perthes' was 7 years and 8 months (range 3 to 12 years). The duration of symptoms was calculated and this was taken into consideration to calculate the probable age of onset of the disease- the reason being that most of our cases had already undergone treatment elsewhere prior to coming here. The mean probable age of onset of disease was 7 years and 2 months. (Three children presented six months after onset of symptoms, 3 children presented eight months after onset of symptoms, one child presented 3 years later, one child presented 3 years and eight months later, the rest presented within 2 months of onset of symptoms). The distribution of cases according to age of onset of disease is given opposite.

MALE: FEMALE RATIO

There were seventeen boys and three girls in our study. The findings on our study showed a male:female ratio of 5.6:1.

GEOGRAPHICAL LOCATION

The geographical location was mapped on both the map of India and in the state map of West Bengal as most of our cases were from there.

STAGE - MODIFIED ELIZABETH TOWN STAGING

Most of our cases presented to us in stage 2A(35%). 20% of our cases had already reached the stage of reossification when they sought treatment with us.

HERRINGS CLASSIFICATION

We had classified Herrings C as severe disease and 12 of our cases presented with Herrings C. Six of our cases had Herring B of which 3 progressed to Herring C during the course of treatment here. Two cases with herring A on presentation had progressed to grade C disease during the course of treatment.

SALTER THOMPSON CLASSIFICATION.

Only six had a clearly defined subchondral fracture line - all these were classified as having grade 2 ST disease. The rest fell under one of the three following categories:-

A- Were operated on before collapse/delineation of subchondral fracture line as decisions were made on the basis of age and degree/severity of involvement

B- Had severe collapse without suchondral fracture

C- Came in Stage 3 disease i.e. when the disease was in the healing phase.

CATERALL GRADING

Sixteen out of twenty one (76%) children had group four disease. Three cases had group three disease and one patient had group one disease. He was classified as severe disease as the collapse of the lateral column was greater than 50%.

HEAD AT RISK SIGNS

Gage Sign

Eleven of the twenty cases had gage sign positive. Six had no gage sign. Three cases were in the healing stage and therefore could not be commented on.

Lateral extrusion

Twelve cases had lateral extrusion, 8 did not. Some of these cases developed lateral extrusion that became obvious after surgery.

Horizontal growth plate

Eleven of our cases had a horizontal growth plate on X-rays. Eight cases had no horizontal growth plates, in one patient it was difficult to comment on the growth plate as he came in stage III B.

All those cases who had a horizontal growth plate also had lateral extrusion except three i.e. if there was an adduction deformity there was very likely a lateral extrusion of the head as well.

Metaphyseal cysts

All cases defined as having severe disease had metaphyseal cysts except two - the severity of disease correlated well with the incidence of metaphyseal cysts – suggesting that there was severe involvement of the epiphysis. If metaphyseal cysts are visible, it is very likely that the patient had a severe disease.

Three of the cases had only one head at risk sign. All the others had atleast two. (two signs – seven cases, three signs- five cases, four signs- 5 cases.)

Trauma and infection

Only three cases had a definite history of trauma (14%). Three other cases had a definite history of fever. One patient had a history of rheumatoid arthritis that was diagnosed after the diagnosis of Perthes' was made. She was started on steroids eventually.

Thrombophilia testing

Protein C

The normal values of Protein C levels are 50-150% . All values were normal and there was no significant difference between cases and controls. The values of Protein C had skewed (non normal) values and therefore were evaluated using the Mann-Whitney U tests. The mean value for protein C in cases and controls was 92.3 and 123 with SDs of 16.5 and 122 respectively. The difference between the two was not significant.(p value of 0.26)

Protein S

The normal values of Protein S total are between 60-140% and the values of free Protein S are between 50 – 150%.

Protein S total and Protein S free were evaluated in the cases first. The protein S total values were found to be normal and therefore controls were not done. The protein S free values were evaluated in both cases and controls and there was no significant difference between them. The value for protein S total was 89.3 ± 17.9 in cases. The values for Protein S free was 88.68 ± 32.4 in cases and 98.34 ± 43.8 in controls.

There was difference in the values between the controls and the cases when the two tailed t-test was used but this was not significant (P=0.421).

Antithrombin (AT)

The normal values for AT are between 80-120%

The values for the controls was 131.58 ± 12.2 and the values for the cases was 118.3 ± 26.8 . There was a significant difference between the two (p= 0.03).

Sickle cell and Paroxysmal nocturnal Haemoglobinuria.

None of the cases or controls tested positive for either of these.

Fibrinogen

The normal values for fibrinogen are 150-450%. The mean value in cases was 267.5 % with a SD of 41.5.There were no values above 350 and none below 200. There was not a single patient who was an outlier- and hence no controls were done.

Factor VIII

The normal levels for F VIII is between 50-150%.

The values of Factor VIII had skewed (non normal) values and therefore were evaluated using the Mann-Whitney U tests. The mean values of FVIII for the cases was 190.2 (SD – 116.4) and the controls was 114.2 SD – 36.5). There was a highly significant difference between the two with a P- value of 0.002.

Eleven (Fifty five percent) out of 20 cases had a value above 150% as against only 3 (13%) of 23 in controls. Using a cutoff of 150% as normal, a two tailed analysis was done using the Fishers exact test and the difference was significant with a p value of 0.008. The odds ratio of a person with Perthes' disease having a FVIII value above 150% was 8.14 (95% confidence interval of between 1.9-36.5)

Activated Protein C resistance (APCR) and Factor V Leiden mutation

Activated protein C values are calculated using the gold standard – as the time taken by a person with a homozygous mutation of Factor V Leiden mutation in the test. The values in our lab were

1.2 to 2 with an absolute value of 2 being taken as the cut of value for normal thrombosis. The mean value for the controls was 1.96 with a SD of 0.237 and mean for the cases was 2.95 and a SD of 0.46.

These two variables are interconnected as the FVL mutation is the commonest cause for APRC. There are undetected causes for APRC as well as other uncommon mutations that affect APRC. There was a significant difference between the values of the cases and controls. The values for the cases was lower in the controls than the cases. (1.96 vs 2.96, standard dev of 0.23 and 0.46 respectively.)

Prothrombin 20210 mutation

There were no positives in either of these in both the controls and the cases.

MTFHR mutation

Three cases had the MTFHR 677 mutation of which one was homozygous for the mutation and two were heterozygous for the mutation. None of the controls were positive.

Anti Phospholipid antibody syndrome

Three of the cases had a mild positive DRVVT test for anti phospholipid antibodies

Risk factor analysis

All the cases with any positive risk factor for thrombosis were evaluated separately. Cases were analysed for atleast one risk factor - thirteen of the cases had at least one positive risk factor for thrombosis (65%). Only 2 of the controls had any risk factor for thrombosis. The Fishers exact test (two tailed) was done to analyse the odds ratio for this and there was a highly significant (p- 0.0002) chance that the children with Perthes' disease would have a concomitant risk factor for thrombosis. The Odds ratio for having a risk factor among children with Perthes' disease was 19.5 (95% CI of 3.5 – 108.5).

Combined Risk factor analysis

Twenty five percent of all the cases had atleast 2 independent risk factors for thrombosis versus none in the control group. This is significant as there is an additive effect for thrombotic factors on the incidence of thrombosis itself.

DISCUSSION

Perhaps, then the time is ripe for us to turn full circle and recall two questions which Legg (1910) asked himself about this disease. “Is this condition the result of congenital deformity or faulty development? Is it the result of a constitutional disease?”

R. Geoffrey Burwell EX49

…but one thing that we need to change
is the one thing that we can change and that
is the eventual shape of the femoral head.

Salter RB. EX103

Discussion

Perthes' disease is at present considered to be of multifactorial origin. [89.] In India very little work has been done on the epidemiology or etiology of Perthes'. Most of the work done on Perthes' disease has been done in Manipal in the Konkan region by Chacko et al , Joseph et al and Rao et al [91, 85, 17, 24 , 101]. They have shown that this disease occurs in the western region of India, in the coastal belt, with an incidence of 4.4/100,000/year. The male:female ratio as reported by Chacko et al was 2.58:1 and the age at onset of disease was 10 years [24]. There is a retardation of growth in these children with Perthes' disease that was most pronounced in the feet.[95,] Joseph et al noted that about 50% of their cases had severe disease [91,]. However, there has been no investigation so far on the etiology of Perthes' disease in India except in relation to the disturbance in growth, immunoglobulin assay [85,] and rural–urban differences [95,]

Data from the other countries has implicated somatomedin levels, immunoglobulin levels and thrombophilic factors such as protein C, protein S, hypofibrinolysis, Antiphospholipid antibodies, antithrombin deficiency and factor V mutation. ([77, 85,], [86,], [10, 2, 1, 3]) . There have been an equal number of studies that refute the involvement of the above in causation of Perthes' [5, 4, 6,7].

A number of experimental studies carried out on dogs show that arterial and venous thrombosis can reproduce the histological and radiological picture of the bone infarct seen in Perthes' disease(EX30, [62,61,69]). It has been categorically demonstrated that a single episode of thrombosis does not lead to changes of Perthes' disease in animals. The effect of the first infarct is often transient. The second infarct is required to produce the characteristic radiological collapse which occurs in Perthes' disease. The observations on the human Perthes' histology using core biopsies showed multiple infarcts in various stages of healing occurring in the same femoral head, indicating that it is, indeed, the result of recurrent insults [26]. Such a picture fits in with a systemic pathology rather than a local insult of traumatic etiology. Venous or arterial occlusion can be caused by thrombophilia due to smoking, PNH, autoimmune disease and anti phospholipid antibody syndrome, obesity, immobilization, and dysfibrinogenemia among other causes ([58,60]). A genetic basis for thrombosis has been considered in Perthes' disease, however, findings such as the male predominance have baffled investigators. Till recent years, other than indirect observation and statistical models, there was little scope to prove the

genetic basis for Perthes' disease. Since the occurrence of Perthes' disease in families does not fit in to the inheritance pattern that is classical of a recessive or dominant single gene transmission, it follows that Perthes' must be of multifactorial origin. The low incidence of Perthes' in twins points to another contributory cause. This, then, can be explained only by both a genetic as well as a local pathology.

In Perthes' disease, the femoral head is susceptible to insult by a thrombotic phenomenon more than any other site because of its peculiar blood supply and its temporal change in the growing child. Below 5 years, the blood supply of the femoral head is almost entirely derived from the transphyseal and the lateral epiphyseal vessels. At around five years, the physeal vessels undergo attrition and the supply is derived mainly from the lateral epiphyseal vessels, except for a minor contribution from the medial metaphyseal vessels. From five to nine years, the head is at risk as only the lateral epiphyseal vessels contribute any blood supply of significance. At nine years, the vessels of the ligamentum teres begin to grow and provide a significant contribution near skeletal maturity. At skeletal maturity, the physeal plate disappears and there is an additional intramedullary supply to the head of the femur. ([63], [65], [28]) In Perthes', it has been shown that the growth rate is decreased, in the preadolescent period when the child is affected by the disease. [75 95,] It is possible that this delay in growth mirrors a similar delay in the evolution of the blood supply of the femoral head. Children with this slowed growth pattern due to any cause may have an increased susceptibility to AVN and therefore to Perthes' disease.

Effusion in the hip joint can occur due to many causes. Trauma, transient synovitis, infection, reactive arthritis, hemophilic arthritis [82, 83,84.] are some of the causes seen in the paediatric age group. In addition, the unyielding capsule of the hip in the extended position increases the pressure and further compromises the blood supply when there is an effusion [62,61], [28].This forms the third contributory factor in our understanding of Perthes' disease etiology. It appears that the presence of more than one factor would precipitate the infarction of the femoral head.

This study has tried to look at thrombophilia, a major contributory factor in Perthes' disease. As mentioned earlier, there has been divided opinion as to the contribution of thrombotic factors. None of the studies that have been done have examined all thrombotic factors comprehensively. The understanding of the role of factor eight in thrombophilia has come about only in recent years, and this

has not been looked at in past studies. We have looked at all known thrombotic factors except TAFI (thrombin activable fibrinolysis inhibitor), and Tissue plasminogen activator inhibitor. There is a difference in the incidence of thrombophilic factors in the various populations as discussed previously. We believe that the reason why studies have negated the association between Perthes' disease and thrombophilia is because a comprehensive screen was not done. [2], [3], [4,5], , [6,7], [8,9],

The importance of investigation into the role of thrombophilia in Perthes' disease cannot be underestimated- as an understanding of this etiology is likely to open up new treatment avenues both to prevent disease, as well as to arrest progression of disease. As of now, investigations for transient synovitis investigation do not include the study of thrombotic factors. A repeat attack of transient synovitis in a patient with a previous history could be the precipitating event that causes the second infarct resulting in Perthes' disease. So, to prevent Perthes' disease, action will need to be taken at or during the first attack of synovitis. Investigating for Thrombophilia during the first attack of synovitis could provide us with a window period to treat patients before they develop flagrant Perthes'. The identification of this '***pre Perthes'*** stage would allow us to formulate a preventive plan.

This study focussed on thrombophilia but also made an attempt to define the distribution of population. More than 50% of our children with Perthes' disease came from West Bengal (11/20). The previous reported areas of high incidence in India are the Konkan region and Kerala. Our mapping reveals a probable focus in the east coast of India like that on the West coast as reported by Joseph et al. [17] To conclusively prove a high incidence in this area would require a population based study.

The mean age of our patients at onset of disease was 7 years and 8 months and the age of onset of symptoms was 7 years and 2 months. This is higher than that reported in the western literature, ex103, [18,19,75.], but lower than that reported from India [91,]. It is possible that there are regional differences between the west coast and the east coast of India because of difference in the etiological cause.

Our boy:girl ratio of 5.6:1 was close to the range quoted by most studies [24 ,17,25,18,19,20,23,22,]. The male predominance of Perthes' disease has not been satisfactorily explained before. Factor VIII elevation was implicated as a cause for thrombosis only in 1994. Factor VIII has been shown to be an independent risk factor for thrombosis and that there is familial clustering of cases with high FVIII levels.

[36], [39]). In our study, 11 of the 17 boys had elevated factor VIII levels. Factor VIII elevation was first implicated as a cause for thrombosis in 1995. [39] The previous studies on Perthes' disease looking for thrombotic etiology have not studied the effect of this factor and therefore its role has not been documented so far. This is a major finding in our study that has not reported in the literature in association with Perthes'. [25],[2],[1,3,7,4,] In our study there is a highly significant difference in the values of FVIII between patients and controls. (p- 0.0002). The odds ratio of a child having an elevated FVIII in our children with Perthes' disease was 8.14(95%CI of 2-36). The observation that FVIII was present in 11 boys out of 17 and none of the three girls was not statistically significant with a p-value of 0.07. This is probably due to the fact that there were very few girls in our study. Since Factor VIII gene is coded on the X chromosome, we are able to postulate that a male predominance is probably based on an X-linked genetic abnormality. This could then explain the high boy:girl ratio recorded in all studies.

Fifty five percent of our patients came from West Bengal (11/20). The others were distributed among Tamil Nadu, Jharkhand and Kerala. Coastal Kerala has been identified as an area having a higher incidence. The greatest number of patients outside of West Bengal has been from Vellore inspite of the low incidence previously reported from this area. It is possible that there was a low rate of diagnosis in the past. A school survey in the community is required to asses the true incidence of the disease in Tamil Nadu. That a large proportion of patients come from West Bengal and nearby Jharkhand (13/20) raises the possibility of another locus of disease in that region. A larger study is required to assess this. Pockets of high incidence have been reported elsewhere [21,20,]. Such pockets raise the possibility of locally occurring environmental factors such as diet.

Hyperhomocystinemia is one of the factors which lead to thrombosis due to vessel wall damage [32], [33], [34], [35]. It is known that in hyperhomocystinemia administration of vit B12, B6, and folate is known to reduce the levels of homocysteine [35]. Conversely, it is possible that a dietary deficiency of any of these three vitamins may cause elevated levels of homocysteine. Three of our patients had enzyme MTHFR mutations (one homozygous and two heterozygous) that leads to elevated levels of homocysteine in the blood and therefore are known risk factors for thrombosis. In only one previous study has this enzyme mutation been looked for in Perthes' disease. It is a known mutation that has been implicated in arterial

and venous thrombosis in the Indian population and in children. [44,50,]. Our study establishes it as one possible etiological factor that contributes to Perthes' disease in India.

It has been observed that one of the differences between Indian and Western populations is the predominant rural distribution in India. We postulate that one of the etiological agents could be hyperhomocystinemia. A significant part of our rural population is vegetarian and this could influence the levels of Vitamin B6, Folic acid levels and B12 levels in their diet. In the homozygous mutation hyperhomocystinemia is likely. The cases who had the heterozygous mutation will need further investigation i.e. homocysteine levels. Being a case control study, the fact that there were three cases with defects in the MTFHR gene poses a statistical problem as there were no controls with positive results. It is difficult to assess the level of clinical significance of each individual finding as the population values for these mutations are not known.

Antithrombin is a factor which inhibits the action of thrombin on fibrinogen- thus preventing clot formation. Low antithrombin levels would enhance thrombosis [58,60]). One of our patients had a significantly low value of 19.9% - considered to be a significant risk factor for thrombosis. Incidentally, he was also homozygous positive for the MTFHR gene mutation which is a significant risk factor for early arterial and venous thrombosis. There was an elevated antithrombin level in the controls as compared to the patients. (p-value was 0.03). The clinical significance of this is not understood. That the values of children are normally lower than adults is known. If the absolute reduction of the levels is what actually matters, this is of concern as no baseline values for children have been established yet. Current estimates of values in children have been estimated using extrapolation. The importance of this data will need to be examined from the viewpoint of the relative levels of other clotting factors – a further population based study is needed.

The major work on thrombophilia in Perthes' disease has been done by Glueck et al. Their principal findings were that up to one-third of 64 children with this disorder had resistance to activated protein C (APC), currently the commonest reported cause of inherited thrombophilia. However, only a half of the children with APC resistance, 12 in all, had a confirmatory polymerase chain reaction (PCR) to show that resistance was due to the presence of the factor V Leiden (FVL) mutation. Eight had the mutation with one homozygote. In the control group the prevalence of this factor was only 1%. In a later

paper, the same group showed that the 'standard' APC resistance assay used to screen for the FVL mutation was a poor predictor of the mutation. Ideally, a 'modified' assay should be used which adds factor V deficient plasma and therefore dilutes out other abnormalities which may affect the activated partial thromboplastin time. An additional finding was an apparently 'low' protein-C antigen level in 28% of the 64 patients studied by Glueck et al but the authors did not use age-adjusted normal ranges for protein C. These take account of the normal physiological deficiency which is present until adulthood and their use would have diminished the number of patients with apparent low levels. [1 55],

` Though the activated protein C resistance is the commonest cause and the factor V Leiden the commonest mutation discovered to cause thrombosis in the western population, there were no significant differences in the values for protein C between patients and controls in our study. The value of Protein C is known to be lower in children and reach adult levels approximately by age 11[47].Protein S Values were also normal in our patients. This indicates that there may be a wide variation in the causes leading to thrombosis in different populations and that each population needs to be investigated separately.

None of the other factors examined were positive. The tests for sickle cell , Paroxysmal nocturnal haemoglobinuria, Factor V Leiden mutation and the Prothrombin 20210 gene mutation were negative in both the patients and controls. The fibrinogen levels were also within normal levels.

Anticardiolipin antibodies have been associated with adult osteonecrosis. [57]. There is no reason to suppose that there is a difference in thrombotic factors causing Perthes' in children and avascular necrosis in adults. In our study population of 20 cases, there were three children who had tested positive with the DRVVT. The DRVVT measures the significance of antiphospholipid antibodies. Though the significance of an isolated DRVVT positive result is doubtful, the fact that all those who tested positive also had another risk factor as well is significant (all of them had concomitantly high values of FVIII).

Thrombosis is a multicausal disease and the risk of thrombosis increases synergistically with the addition of risk factors. For example, the annual risk for women with the factor V Leiden who did not use oral contraceptives was 5•7 per 10 000 people (relative risk 6•9), that for women who used oral contraceptives but did not carry factor V Leiden was 3•0 per 10 000 women (relative risk 3•7), and that

for women with factor V Leiden who used oral contraceptives was 28•5 per 10 000 people (relative risk 34•7). [58]. In this study on severe Perthes' disease (Caterall III or IV /Herrings C), 25% of our patients had more than one risk factor indicating that there is a strong additive effect in Perthes' as has been described in venous thromboembolism. In our study 13 out of 20 children had atleast one positive risk factor as compared to two of the controls. Children with Perthes' disease had an odds ratio of 19.5 of having a risk factor for thrombosis and using the Fishers exact test- two tailed, there was highly significant (p - 0.0002) association between the two. Twenty five percent of our cases had two independent risk factors for thrombosis as compared with none of the controls and the significance of this cannot be underestimated as the effect of two risk factors is synergistic. This proves that in Indian children with Perthes' disease, thrombophilia is highly likely. The factor levels of atleast one of our children is significant enough to require treatment immediately. There are no clear cut guidelines as yet on the treatment of Perthes' with antithrombotic drugs. A large scale or multicentre study is indicated to establish guidelines for the investigation and management protocols in the 'pre-Perthes'' stage.

Three of our patients had a history of transient synovitis of the hip. A synovitis or effusion of the hip is implicated in the etiology of Perthes' disease. A common problem in our patients is a reactive arthritis of the hip often caused by upper respiratory or GI infection. Potable drinking water is not available to most of the rural population in India. If enterically acquired reactive arthritis is taken to be one of the causes for reactive arthritis / 'transient synovitis' (a postulated mechanism for Perthes' disease) in the hip, it could explain the higher incidence in rural areas and in areas with the highest deprivation scores. EX 4, [21] Viral infective / reactive arthritis may also explain the urban clustering in studies from Liverpool and northern United Kingdom. It has been shown that one episode of synovitis predisposes a child to further attacks. There is evidence to support this in terms of elevated immunoglobulin levels in studies both from India and elsewhere [85, 86,]). Thus, in the susceptible age group and the predisposed child, reactive arthritis is a risk factor for Perthes' disease. Transient synovitis due to trauma may also be a contributing factor for the disease. There are two problems with this, one that there often is a significant recall bias associated with trauma, and two, that in the young child trauma may not be reported at all.

It was noted by Caterall et al [87,] that there was an association between Perthes' disease and congenital urinary tract abnormalities. There is a similar incidence of minor congenital deformities in children with Perthes' disease and those with a single major congenital defect. [89.] This may be a manifestation of a prothrombotic tendency in the child during intrauterine growth that causes both the minor and major defects. We did not see any congenital defects in our patients but the number in our study was too small to evaluate this association.

We conclude that Perthes' disease is a multicausal disease with various etiological factors. Thrombophilia, vascular supply and hip effusion of various aetiologies are probably factors that influence the disease onset and severity, with those that have all these three risk factors being most likely to develop the disease. In our study we have looked for and found a significant number of Patients with thrombophilia, an etiological factor not previously studied in India.

Perthes believed that several different causes could lead to the obstruction of arterial blood flow to the affected femoral head. He and other contemporary surgeons thought that the ailment could arise from several different etiologic factors, operating in different individuals. [15,] Lovell and Winter There probably are other factors that influence the disease such as diet, deprivation and crowding. Our hypothesis takes into account all these factors. We also understand that there are probably more causes for the disease that have not been discovered yet.

CONCLUSIONS

Conclusions

This study establishes Thrombophilia as a major etiological factor in Perthes' disease in India. The factors that were found to be involved were factor VIII, Antithrombin, and antiphospholipid antibody. The genetic mutation involving MTHFR gene was involved.

We propose that studies be carried out to frame guidelines for investigation and management, based on these findings.

BIBILIOGRAPHY

References

1. Glueck CJ, Crawford A, Roy D, Freiberg R, Glueck H, Stroop D. Association of antithrombotic factor deficiencies and hypofibrinolysis with Legg-Perthes disease. J Bone Joint Surg.. 1996;78:3-13.

2. Balasa VV, Gruppo RA, Glueck CJ, Wang P, Roy DR, Wall EJ, Mehlman CT, Crawford AH. Legg-Calve-Perthes disease and Thrombophilia. J Bone Joint Surg [Am]. 2004;86:2642-47.

3. Glueck, CJ; Glueck HI; Greenfield D; Freiberg R; Kahn A; Hamer T; Stroop D; Tracy T. Protein C and S deficiency, thrombophilia, and hypofibrinolysis: pathophysiologic causes of Legg-Perthes disease. Pediat. Res. 1994;35: 383-388, .

4. Kealey WDC., Mayne EE. , McDonald W, Murray P, Cosgrove AP. The role of coagulation abnormalities in the development of Perthes' disease. J Bone Joint Surg [Br]. 2000;82B:744-6.

5. Thomas DP, Morgan G, Tayton K. Perthes' disease and the relevance of thrombophilia. J Bone Joint Surg [Br] . 1999;81-B:691-5..

6. Hresko MT, McDougall PA., Gorlin JB., Vamakas EC. , Kasser JR, Neufeld EJ. Prospective Reevaluation of the Association Between thrombotic Diathesis and Legg-Perthes Disease J Bone Joint Surg [Am]. 2002;84A(9):1613-1618,.

7. S. Hayek, G. Kenet, A. Lubetsky, N. Rosenberg, S. Gitel, S. Wientroub. Does thrombophilia play an aetiological role in Legg-Calvé-Perthes disease? J Bone Joint Surg [Br] . 1999;81B:686-90..

8. Koo KH, Song HR, Ha YC,Kim JR, Kim SJ, Kim KL, Chang KC, Ahn IO, Cho SH. Role of Thrombotic and Fibrinolytic disorders in the etiology of Perthes' disease. Clin Orthop. 2002;399:162-7.

9. Lopez-Franco M, Gonzalez-Moran G, De Lucas JC, Llamas P, de Velasco JF, Vivancos JC, Epeldegui-Torre T. Legg-Perthes disease and Heritable Thrombophilia. J Pediatr Orthop. 2005;25[4]:456-9.

10. Szepesek, Posan E, Harsfalvi J, Azner E, Szucs D., Gaspar L., Csernatony Z., Udvardy M. The most severe forms of Perthes disease associated with the homozygous Factor V mutation J Bone Joint Surg [Br]. 2004;86B, 426-9.

11. Ghosh.K, Shetty S, Madkaikar M, Pawar A, Nair S, Khare A, Pathare A, Jijina F, Mohanty D. Venous thromboembolism in young patients from western India; a study. Clin Appl Thromb Hemost.. 2001;7(2): 158-65.

12. Kumar SI, Kumar A, Srivastava S, Saraswat VA, Aggarwal R. Low frequency of factor V leiden and prothrombin G21210A mutations in patients with hepatic venous outflow tract obstruction in northern India: a case control study. Indian J Gastroenterol. 2005;24:211-15.

13. Leroyer C,Mercier B,Escoffre M, Fe´rec C, Mottier D. Factor V Leiden Prevalence in Venous Thromboembolism Patients Chest . 1997;111:1603-06.

14. Calve J. The Classic: On a Particular Form of Pseudo-Coxalgia Associated with a Characteristic Deformity of the Upper End of the Femur (English translation). Clin Orthop. 1980;150:4-7.

15. Nevelos AB. Perthes' Disease: The Family Tree. Clin Orthop. 1986;209: 13-22.

16. Weinstein SL. Lovell and Winter's Pediatric Orthopaedics. fifth ed. Vol. 2, Piliadelphia: Lipincott Williams & Wilkins;2004. Eds, Morrissy RT Weinstein SL. . ;.

17. Joseph B., Chacko V, Rao BS, Hall AJ. The Epidemiology of Perthes Disease in South India. Internat J Epidem. 1988;17(3):603-7.

18. Barker DJP, Dixon E, Taylor JF. Perthes' disease of the hip in three regions of England. J Bone Joint Surg [Br]. 1978;60(4):478-80.

19. Pillai A., Atiya S., Costigan PS. The incidence of Perthes' disease in Southwest Scotland. J Bone Joint Surg [Br]. 2005;87-B:1531-5..

20. Hall AJ, Barker DJP. Perthes' disease in Yorkshire. J Bone Joint Surg [Br]. 1989;71-B(2):229-33.

21. Barker DJP, Hall AJ. The Epidemiology of Perthes' Disease. Clin Orthop. 1986;209: 89-94.

22. Kealey WDC., Moore AJ., Cook S., Cosgrove AP. Deprivation, urbanisation and Perthes' disease in Northern Ireland J Bone Joint Surg [Br]. 2000;82B:167-71..

23. Rowe S.-M, Jung S-T, Lee K.-B, Bae B.-H, Cheon S.-Y, Kang K-D. The incidence of Perthes' disease in Korea. J Bone Joint Surg [Br]. 2005;87B:1666-8..

24. Chacko V, Joseph B., Seetharam B. Perthes' Disease in South India. Clin Orthop. 1986;209: 95-99.

25. Wynne-Davies R, Gormley J. The Aetiology of Perthes' Disease. Clin Orthop. 1978;60(1):6-14..

26. Inoue A, Freeman MAR,Vernon-Roberts V, Mizuno S. The Pathogenesis of Perthes' Disease. J Bone Joint Surg [Br]. 1976;58-B(4): 453-61 .

27. Tucker FR. Arterial supply at the femoral head and its clinical importance. J Bone Joint Surg [Br]. 1949;31B(1):82-93 .

28. Atsumi T, Yamano K, Muraki M, Yoshihara S, Kajihara T. The Blood supply of the lateral epiphyseal arteries in Perthes' disease. J Bone Joint Surg [Br]. 2000;82B:392-8.

29. Anderson FA Jr, Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, Forcier A, Dalen JE. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern med.. 1991;151(5):933-8. .

30. O'Donnell J, Mumford AD, Manning RA,Laffan M. Elevation of FVIII:C in Venous Thromboembolism Is Persistent and Independent of the Acute Phase Response. Thromb Haemost . 2000;83:10–3.

31. Koster T, Rosendaal FR, de Ronder H, Brier E. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet. 1993;342:1503-1506.

32. Jakubowski H. Homocysteine Is a Protein Amino Acid in Humans. Jour Biol Chem. 2002;277(34):325–328, .

33. Thambyrajah J, Townend JN. Homocysteine and atherothrombosis — mechanisms for injury. Eur Heart J. 2000;21(12):967–974.

34. Austin RC, Lentz SR, Werstuck GH. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death and Differentiation . 2004;11:56–64.

35. D'Angelo A, Selhub J. Homocysteine and Thrombotic Disease. BLOOD. 1997;90(1):1-11.

36. Schambeck CM, Hinney K, Haubitz I, Mansouri B, Taleghani D, Keller WF. Familial Clustering of High Factor VIII Levels in Patients With Venous Thromboembolism. Arterioscler Thromb Vasc Biol. . 2001;21:289-292..

37. Kyrle PA, Minar E, Hirschll M, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med . 2000;343: 457–62..
38. O'Donell J, Tuddenham EGD, Manning R, et al. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction. Thromb Haemost . 1997;77: 825–8.

39. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. . Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet.. 1995;345:152–155..
40. Tirado I, Mateo J , Manuel-Soria J , Oliver A , Martínez-Sánchez E , Vallvé, Borrell M , Urrutia T, Fontcuberta J. The ABO blood group genotype and factor VIII levels as independent risk factors for venous thromboembolism. Thromb Haemost . 2005;93: 468-74.
41. Dossenbach-Glanninger A, Trotsenburg Mv, Krugluger W, Dossenbach MR, Oberkanins C, Huber J, Hopmeier P. Elevated coagulation factor VIII and the risk for recurrent early pregnancy loss. Thromb Haemost . 2004;91: 694–9.
42. Kupferminc MJ, et al.. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med . 1999;340(1): 9-13..
43. Kahn S, Rey E, David MM. Factor V Leiden and fetal loss: a meta-analysis by type and timing of fetal loss. Blood . 2001; 98(11): 50a..
44. Shah Sudeep R., DasGupta A, Sharma A, Anand J, Devendra D, Phillip A, Pravin R, Mukta B. Thrombophilic conditions in Non-cirrhotic portal vein thrombosis. Indian J Gastroenterol. 2005;24[5]:205-10.
45. Kraaijenhagen RA, in't Anker PS, Koopman MMW, Reitsma PH, Prins MH, van den Ende A, Buller HR. High Plasma Concentration of Factor VIIIc Is a Major Risk Factor for Venous Thromboembolism. Thromb Haemost . 2000;83: 5–9.
46. Kamphuisen PW, Eikenboom JCJ, Vos HL, Pablo R,Sturk A, Bertina RM,Rosendaal FR. Increased Levels of Factor VIII and Fibrinogen in Patients with Venous Thrombosis Are not Caused by Acute Phase Reactions. Thromb Haemost . 1999;81:680–3.

47. Karpatkin M, Manuccio-Manucci P, Bhogal M, Vigano S, Nardi M. Low protein C in the neonatal period. Br Jour Haemat.. 1986;62:137-42.
48. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci U S A. 1993;90:1004.
49. Bertina RM, Koelman BPC, Koster T. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-67.
50. Chan AK, Deveber G, Mongale L, Brooker A , Massicotte PM. Venous thrombosis in children. J Thromb Haemost. 2003;1(7):1443-55.
51. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med. 1995;332:912-917.
52. Hooper WC, Dilley A, Ribiero MJ, A racial difference in the prevalence of the Arg506->Gln mutation. Thromb Res. 1996;81:577-581.
53. Mannucci PM, Vigano S. Deficiencies of protein C, an inhibitor of blood coagulation. Lancet. 1982;2(8296):463-7.
54. Eldridge J, Dilley A, Austin H,EL-Jamil M, Wolstein L, Doris J, Hooper C, Meehan PL, Evatt B. The Role of Protein C, Protein S, and Resistance to Activated Protein C in Legg-Perthes Disease. Pediatrics. 2001;6:1329-1334 .
55. Glueck CJ, Brandt G, Gruppo RA, Crawford AH, Roy DR, Trent T, Stroop D, Wang P, Becker A. Resistance to Activated Protein C and Legg-Perthes disease. Clin Orthop. 1997;338:139-52.
56. Arruda VR, Belangero WD, Ozelo MC, Oliveira GB, Pagnano RG, Volpon JB, Annichino-Bizzacchi JM. Inherited Risk Factors for Thrombophilia Among Children with Legg-Calvé-Perthes Disease. J Pediatr Orthop. 1999;19(1):84-87.
57. Korompilias AV, Gilkeson GS, Ortel TL, Seaber AV, Urbaniak JR. Anticardiolipin Antibodies and Osteonecrosis of the Femoral Head. Clin Orthop. 1997;345:174-180.
58. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353(3):1167-73 .

59. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms--the Leiden Thrombophilia Study (LETS). Thromb Haemost. 1994;71(6):719-22 .
60. Bertina RM. Elevated Clotting Factor Levels and Venous Thrombosis. Pathophysiol Haemost Thromb . 2003;33: 399-400.
61. Kemp HBS. Perthes' Disease- The Influence of Intracapsular Tamponade on the Circulation in the Hip Joint of the Dog. Clin Orthop. 1981;156:105-114.
62. Kemp HBS. Perthes' Disease in Rabbits and Puppies. Clin Orthop. 1986;209:139-60.
63. Chung SMK. The Arterial supply of the developing proximal end of the human femur. J Bone Joint Surg [Am]. 1976;58(7):961-70.
64. Trueta J. Normal anatomy of human femoral head and its clinical importance. J Bone Joint Surg [Br]. 1949;31B :82.
65. Trueta J. The normal vascular anatomy of the human femoral head during growth. J Bone Joint Surg [Br]. 1957;39:358-394.
66. Heikkenen E, Lanning P, Suramo I, et. al. The venous drainage of the femoral neck as a prognostic sign of Perthes disease. Acta Orthop Scand. 1980;51(3):501-3..
67. Liu SL, Ho TC. The role of venous hypertension in the pathogenesis of Legg-Perthes disease. A clinical and experimental study. J Bone Joint Surg [Am]. 1991;73A[2]:194-200..
68. Harrison MHM, Burwell RG. Perthes' Disease: A Concept of Pathogenesis. Clin Orthop. 1981;156:115-127.
69. Sanchis M, Freeman MAR, Zahir A. Experimental stimulation of the blood supply to the capital epiphysis in the puppy. J Bone Joint Surg [Am]. 1973;55(2):335-42.
70. Katz JF Recurrent Legg-Calve-Perthes disease. J Bone Joint Surg [Am]. 1973;55(4):833-6.
71. Martinez AG, Weinstein SL. Recurrent Legg-Calve-Perthes disease : case report and review of the literature. J Bone Joint Surg [Am]. 1991;73(7):1081-5.
72. Smith RB, Nevelos AB. Osteochondritis occurring at multiple sites. Acta Orthop Scand. 1980;51(3):445-9..

73. Harrison MHM, Blakemore ME. A study of the "normal" hip in children with unilateral Perthes' disease. J Bone Joint Surg [Br]. 1980;62-B(1):31-6..
74. Kitoh H, Kitakoji T, Katoh M, TakamineY. Delayed ossification of the proximal capital femoral epiphysis in Legg-Calvé-Perthes' disease. J Bone Joint Surg [Br] . 2003;85-B:121-4..
75. Burwell RG., Dangerfield PH, Hall DJ , Vernon CI , Harrison MHM. Perthes' disease- An anthropometric study revealing impaired and disproportionate growth. J Bone Joint Surg [Br]. 1978;60(4): 461- 77.
76. Rayner PHW., Schwalbe SL., Hall DJ. An Assessment of Endocrine Function in Boys with Perthes' Disease. Clin Orthop. 1986;209:124-28.
77. Burwell RG, Vernon CL, Dangerfield PH, Hall DJ, Kristmundsdottir F. Raised Somatomedin Activity in the Serum of Young Boys with Perthes' Disease Revealed by Bioassay. Clin Orthop. 1986;209:129-38.
78. Wynne-Davies R. Some etiologic factors in Perthes' Disease. Clin Orthop. 1980;150;12-15.
79. Hall DJ. Genetic aspects of Perthes disease : a critical review. Clin Orthop. 1986;209:100-14..
80. O'Sullivan M, O'Rourke SK, MacAuley P. Legg-Calve-Perthes disease in a family: genetic or environmental. Clin Orthop. 1985;199:179-81.
81. De Valderrama JAF. The "Observation hip" syndrome and its late sequelae. J Bone Joint Surg [Br]. 1963;45B(3):462-70.
82. Adams JA. Transient synovitis of the hip joint in children. J Bone Joint Surg [Br]. 1963;45B(3): 471-76.
83. Landin LA, Daneilsson LG, Wattsgard C. Transient synovitis of the hip. J Bone Joint Surg [Br]. 1987;69B(2): 238-42.
84. Kallio P, Ryoppy S, Kunnamo I. Transient synovitis and Perthes' disease. J Bone Joint Surg [Br]. 1986;6B(5):808-11 .
85. Joseph B. Serum immunoglobulin in Perthes' disease. J Bone Joint Surg [Br] . 1991;73; 509-10.
86. Matsoukas JA. Viral antibody litres to rubella in coxa plana or Perthes' disease. Acta Orthop Scand. 1975;46:957-62..

87. Caterall, A., Lloyd-Roberts, GC, Wynne-Davies, R. Association of Perthes' disease with congenital anomalies of genitourinary tract and inguinal region. Lancet.. 1971;1: 996-997..

88. Katz, JF. Spina bifida occulta in Legg-Calve-Perthes disease. Clin Orthop. 1959;14:110-117..

89. Hall DJ, Harrison MHM , Burwell RG. Congenital abnormalities and Perthes disease. J Bone Joint Surg [Br]. 1979;61-B(I):18-25.

90. Loder RT, Schwartz EM, Hensinger RN. Behavioural characteristics of children with Legg-Calve-Perthes disease. J Pediatr Orthop. 1993;13(5):598-601.

91. Joseph B, Varghese G, Mulpuri K, Rao KLN, Nair SN. Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset. J Pediatr Orthop. 2003;23:590-600.

92. Salter RB, Thompson GH. Legg–Calve´–Perthes disease. The prognostic significance of the subchondral fracture and a two-group classification of the femoral head involvement. J Bone Joint Surg [Am] . 1984;66:479–489..

93. Herring JA, Kim HT , Browne R. Legg-Calvé-Perthes Disease Part I: Classification of radiographs with use of the modified lateral pillar and Stulberg classifications. J Bone Joint Surg [Am]. 2004;86A(342):1503-1506.

94. Caterall A. The natural history of Perthes' disease J Bone Joint Surg [Br]. 1971;53 B(1):37-53 .

APPENDIX

Proforma

Name Age Sex Hosp No
Age at onset of symptoms
Age at taking blood tests

Address

Have you shifted area of origin?

Veg/Non Veg.

History
Fever

trauma-
High energy/low evergy

Previous treatment

Examination
Anthropometry
Height
Sitting height
Subischial height
Head circum
Upper limb length
Arm length
Forarm and hand
Ll length
Tibia length

Foot length
Biacromial dist
Bi iliac dist
Weight

X ray classification
Stage of disease
Herring’s classification
Salter-thompson classification
Caterall grouping
Head at risk signs
Gage Sign
Lat extrusion
horizontal physis
Lat calcification
Metaphyseal cysts

Thrombophilia work up
Protein C
Protein S total
Protein s free
Antithrombin
Sickle cell preparation
PNH
Fibrinogen
Factor VIII
APCR
DRVVT
Factor V Leiden
Factor II 20210
MTHFR

Diagrams

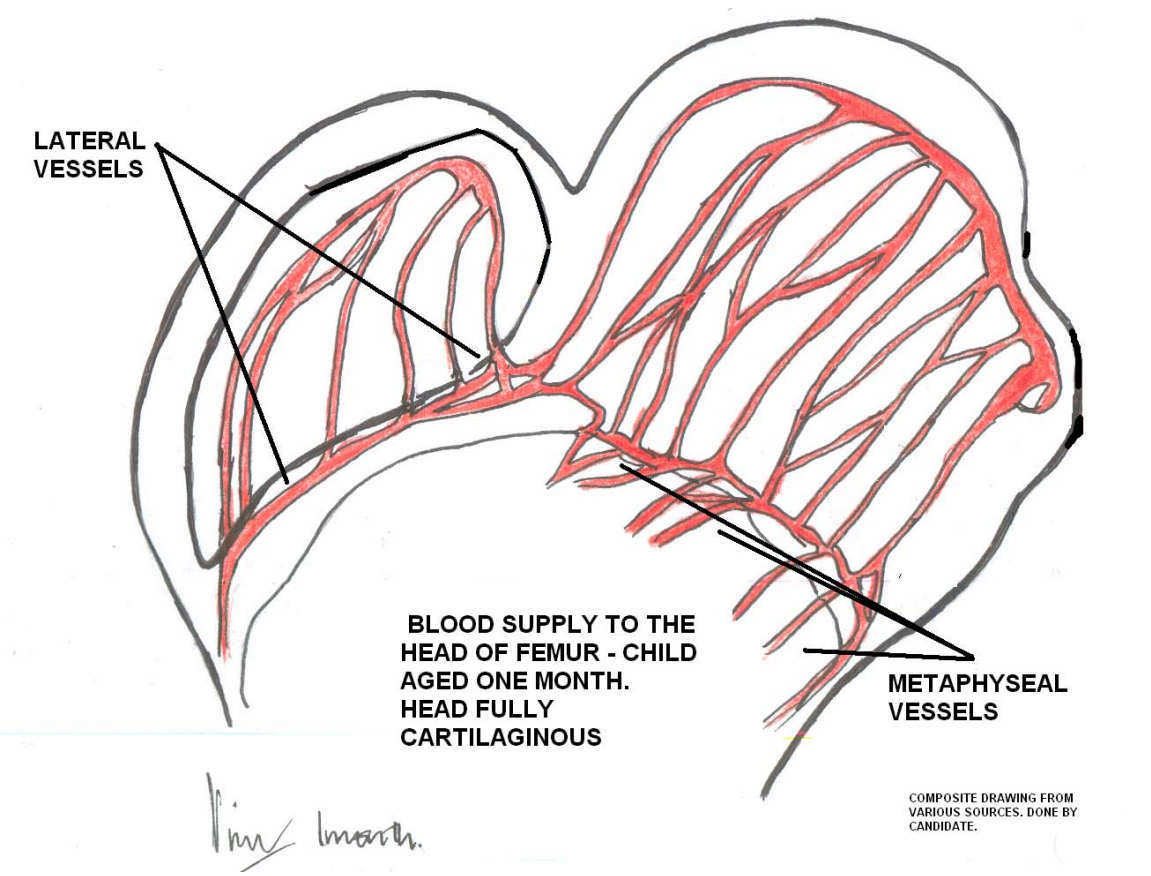


Figure 1. Blood supply to the femoral head in a child aged one month. There is an abundant blood supply both from the lateral as well as the metaphyseal vessels

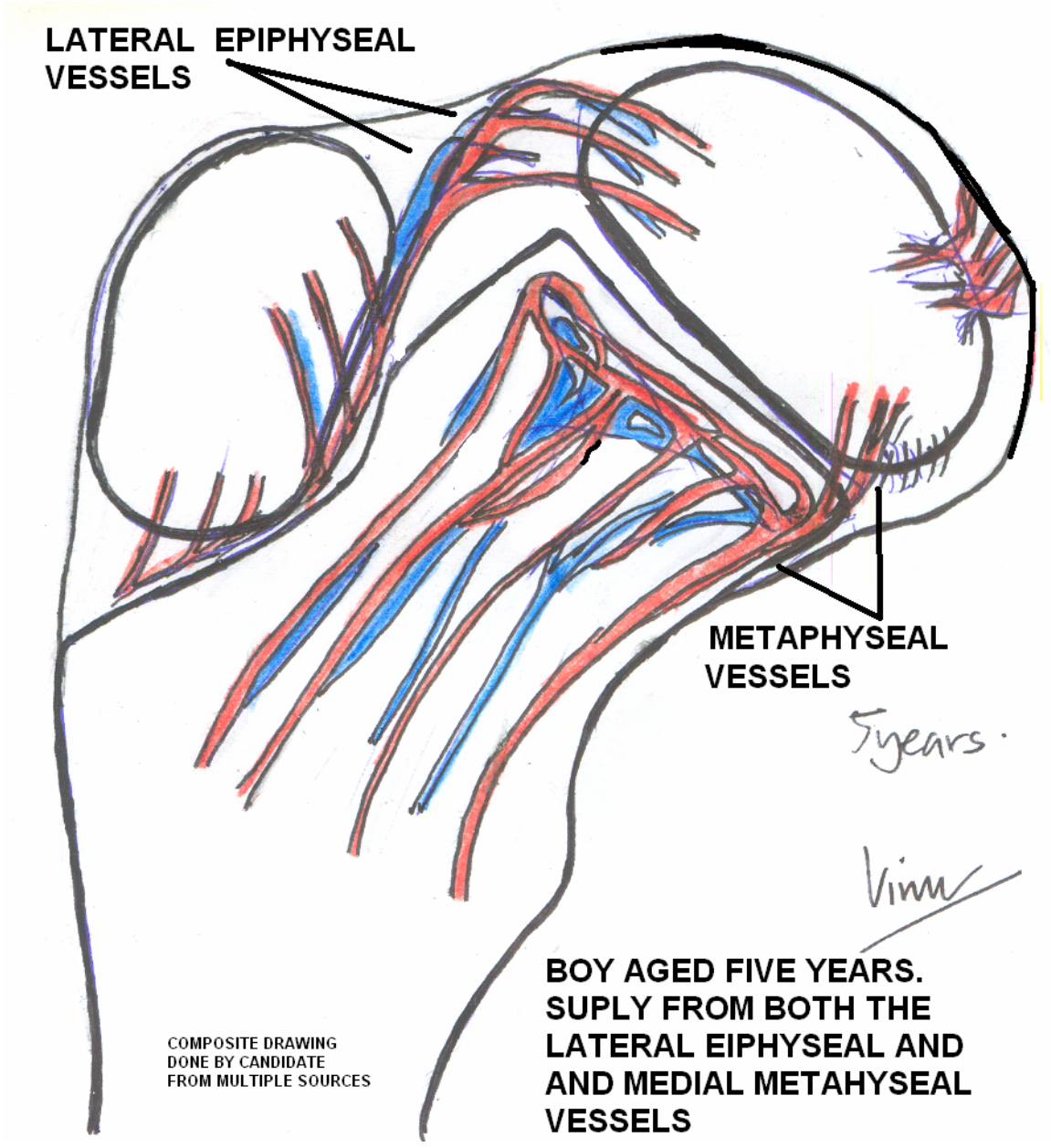


Figure 2. Blood supply to the femoral head in a boy aged five years. The main blood supply is from the lateral vessels. The metaphyseal vessels now contribute only a minor proportion of blood supply. The Ligamentum teres vessels supply the head only at its insertion.

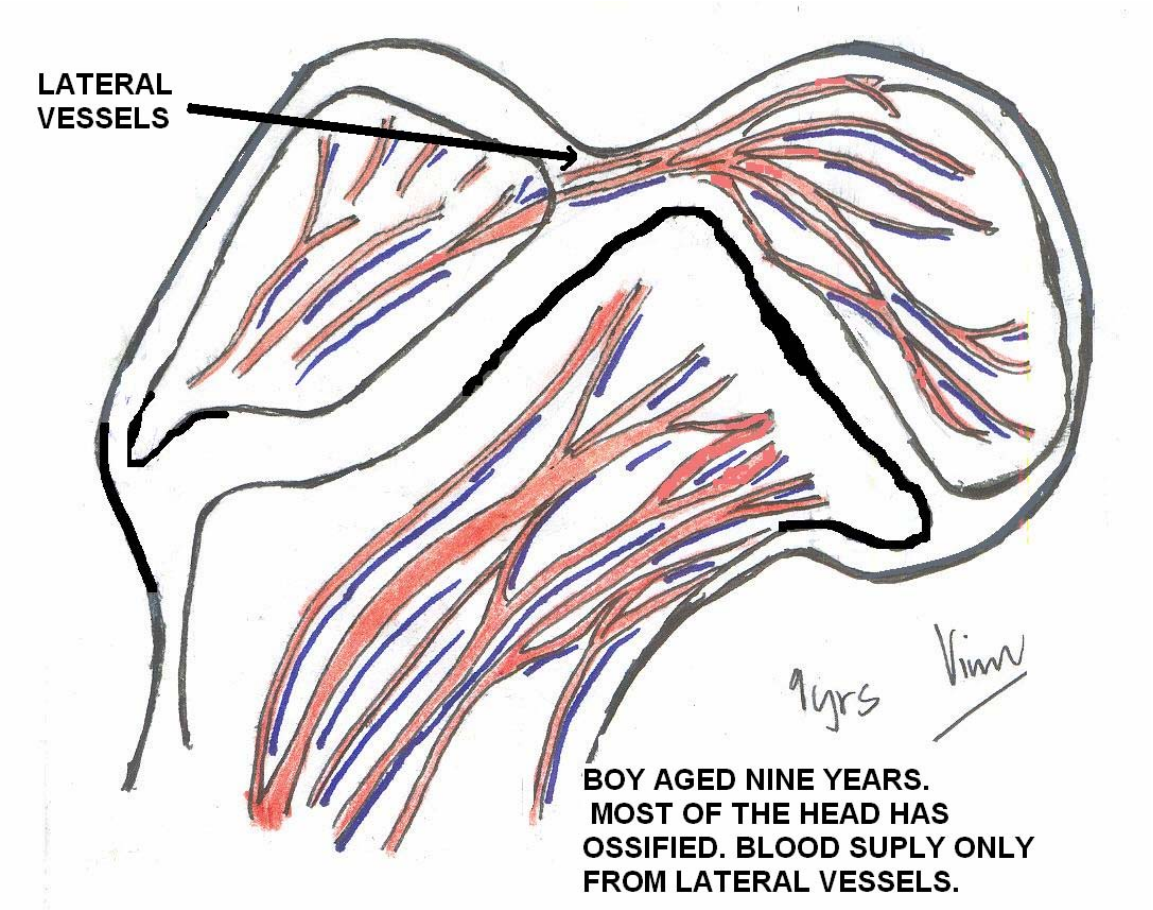


Figure 3. Blood supply to the femoral head in a boy aged nine years. There blood supply solely from the lateral vessels. The metaphyseal are now absent. The ligamentum teres blood supply begins to get established only later.

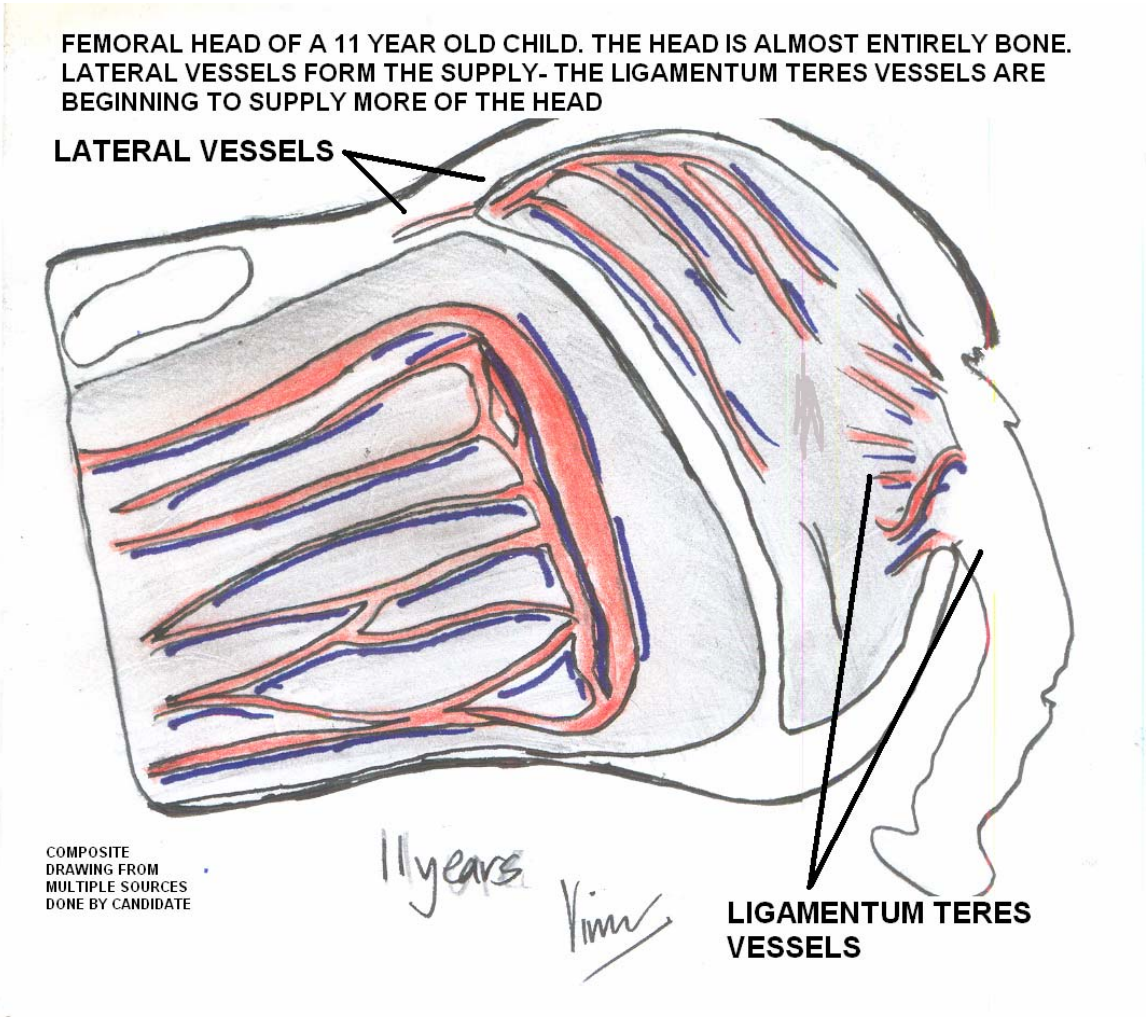


Figure 4. Blood supply to the femoral head in a boy aged 11 years. There blood supply is now in the preadolescent stage The ligamentum teres blood supply contributes a larger portion. When the physeal plate disappears the metaphyseal Blood supply gets reestablished

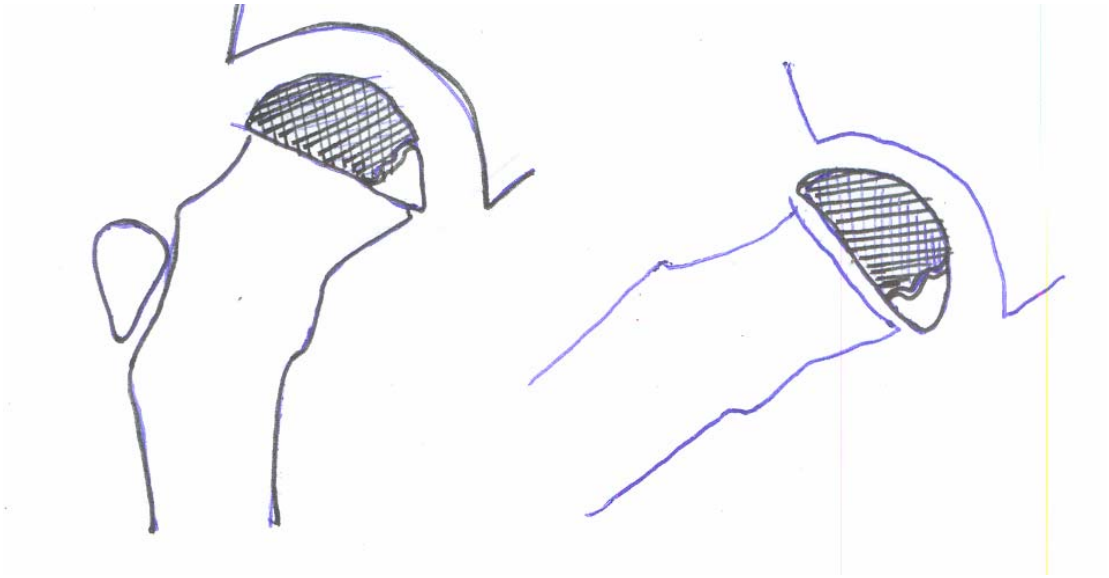


Figure 5. Stage 1A Part or whole of the epiphysis is sclerotic, there is no loss of height. Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr Orthop, 2003; 23:590-600, by the candidate.

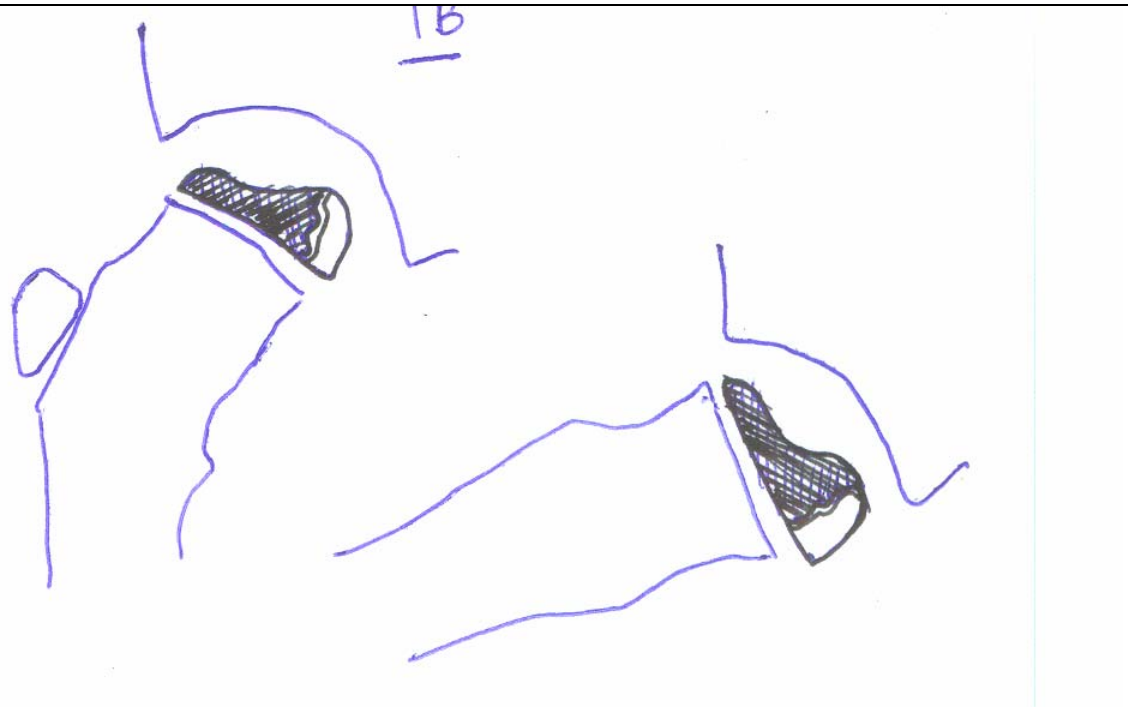


Figure 6. Stage 1B Part or whole of the epiphysis is sclerotic, there is no loss of height. Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr Orthop, 2003; 23:590-600, by the candidate.

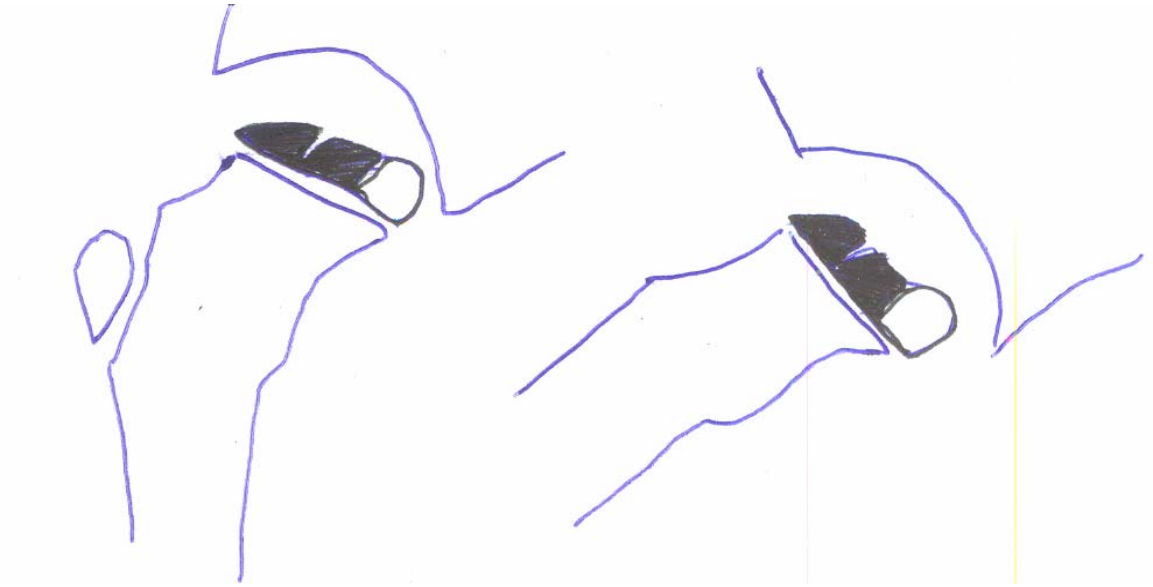


Figure 7. Early fragmentation Stage 2A. Fragmentation has started. There are one ore two vertical fissures. Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr

Figure 8. Late fragmentation Stage 2B: Fragmentation is advanced. There is no new bone visible laterally.

Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr Orthop, 2003;

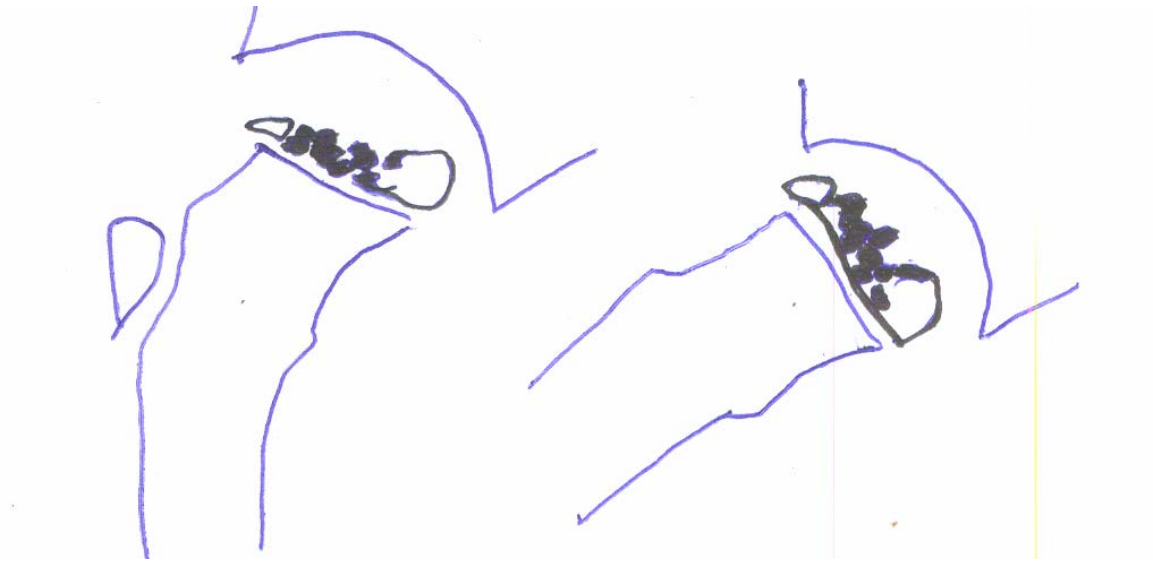


Figure 9. Early reossification-Stage 3A There is new bone visible lateral to the epiphysis, less than 1/3 of the epiphysis is involved, bone is porotic in nature . Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr Orthop, 2003; 23:590-600, by the candidate.

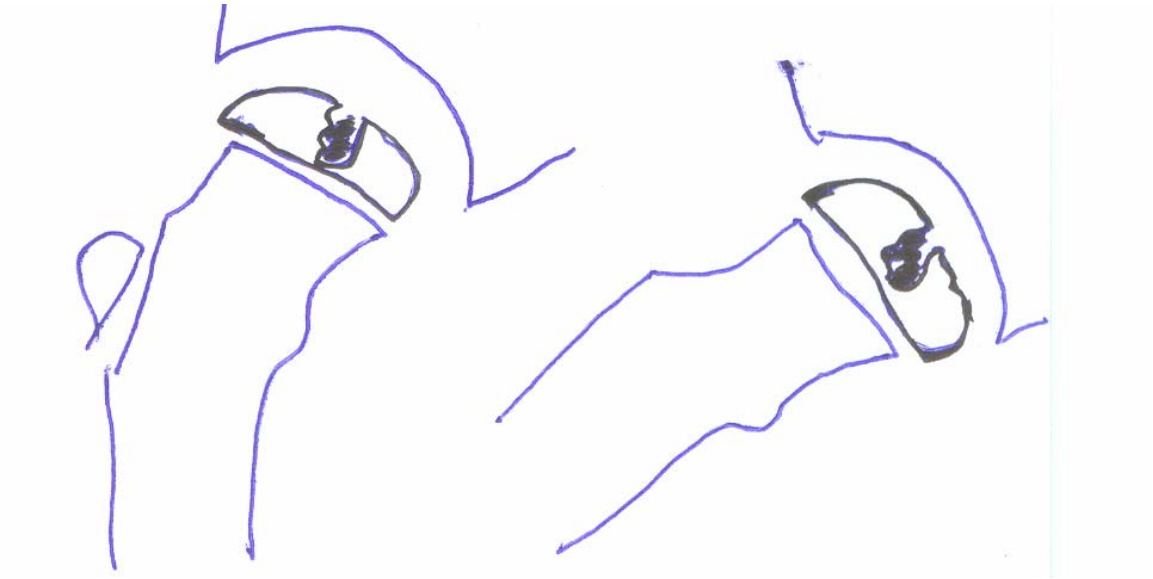
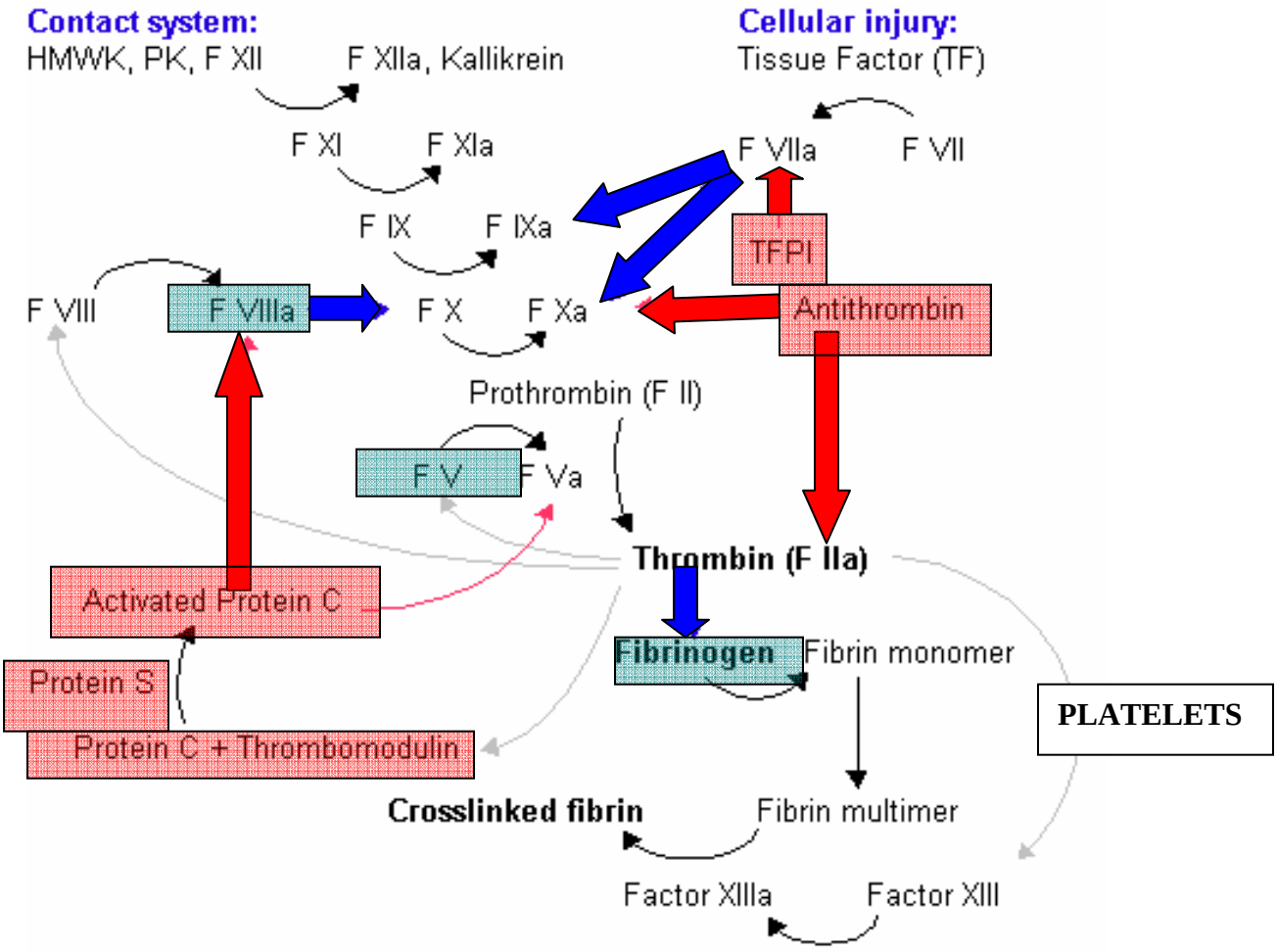


Figure 10. Late reossification-Stage 3B. There is new bone visible lateral and this os of normal texture and more tha 1/3 of the epiphysis. Redrawn from Joseph et al Natural Evolution of Perthes' Disease: A Study of 610 Children under 12 Years of Age ar Disease Onset, J Pediatr Orthop, 2003; 23:590-600, by the candidate.
.

Figure 11:The basic mechanism of the clotting cascade is given below.



The coagulation cascade. Legend: HMWK = High molecular weight kininogen, PK = Prekallikrein, TFPI = Tissue factor pathway inhibitor. Black arrow = conversion/activation of factor. Red arrows = action of inhibitors. Blue arrows = reactions catalysed by activated factor. Grey arrow = various functions of thrombin. Bue – prothrombotic factors. Red – antithrombotic factors.

Figure 12: The functions of the antithrombotic factors are given below.

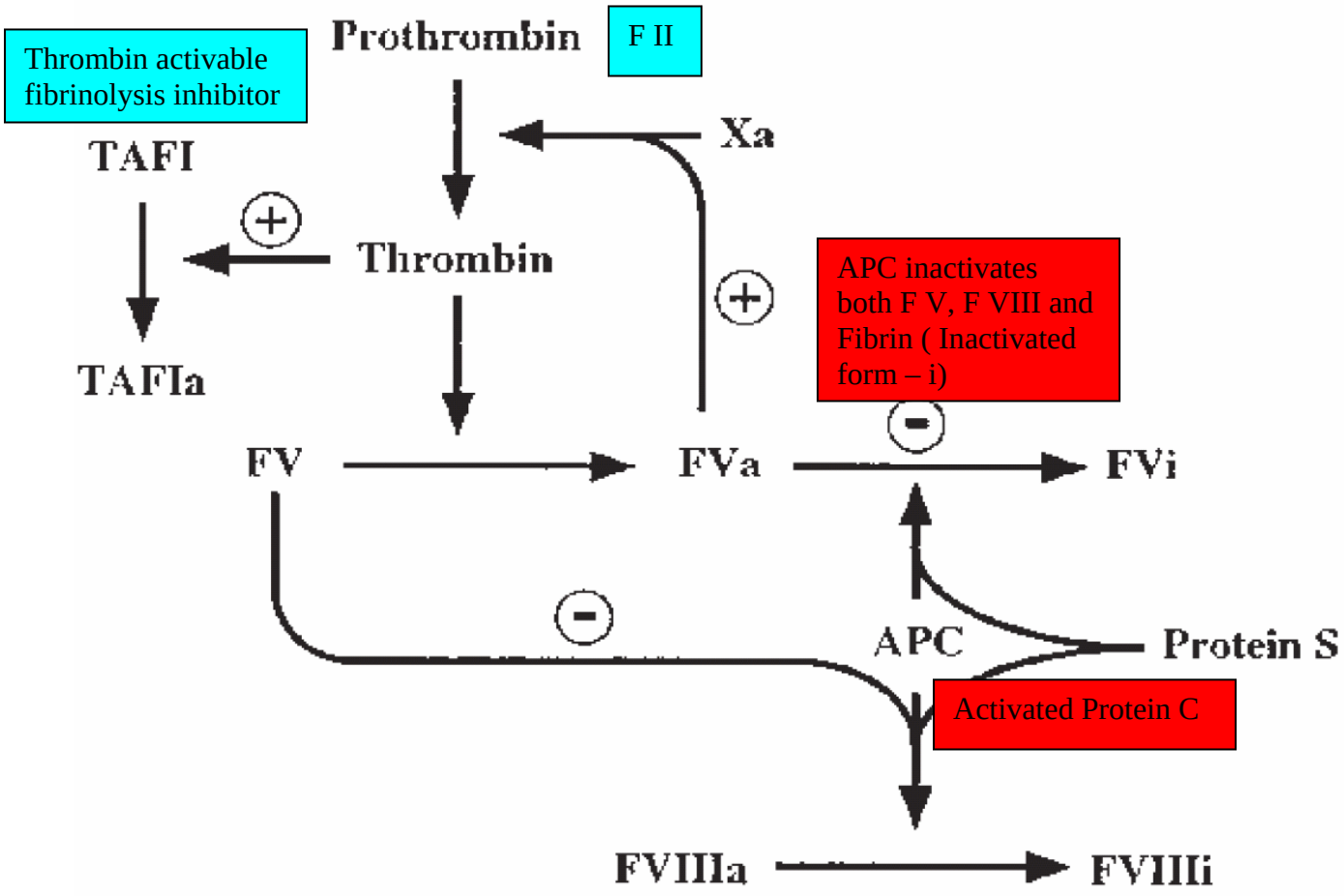
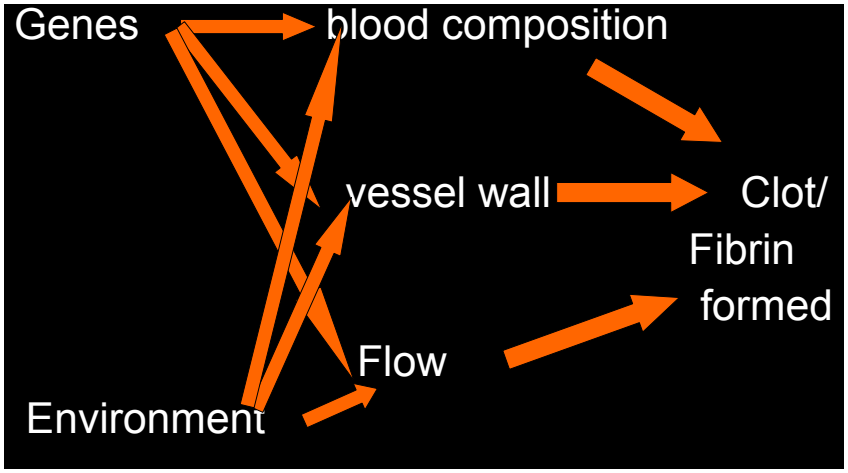


Figure 13: Virchows Triad



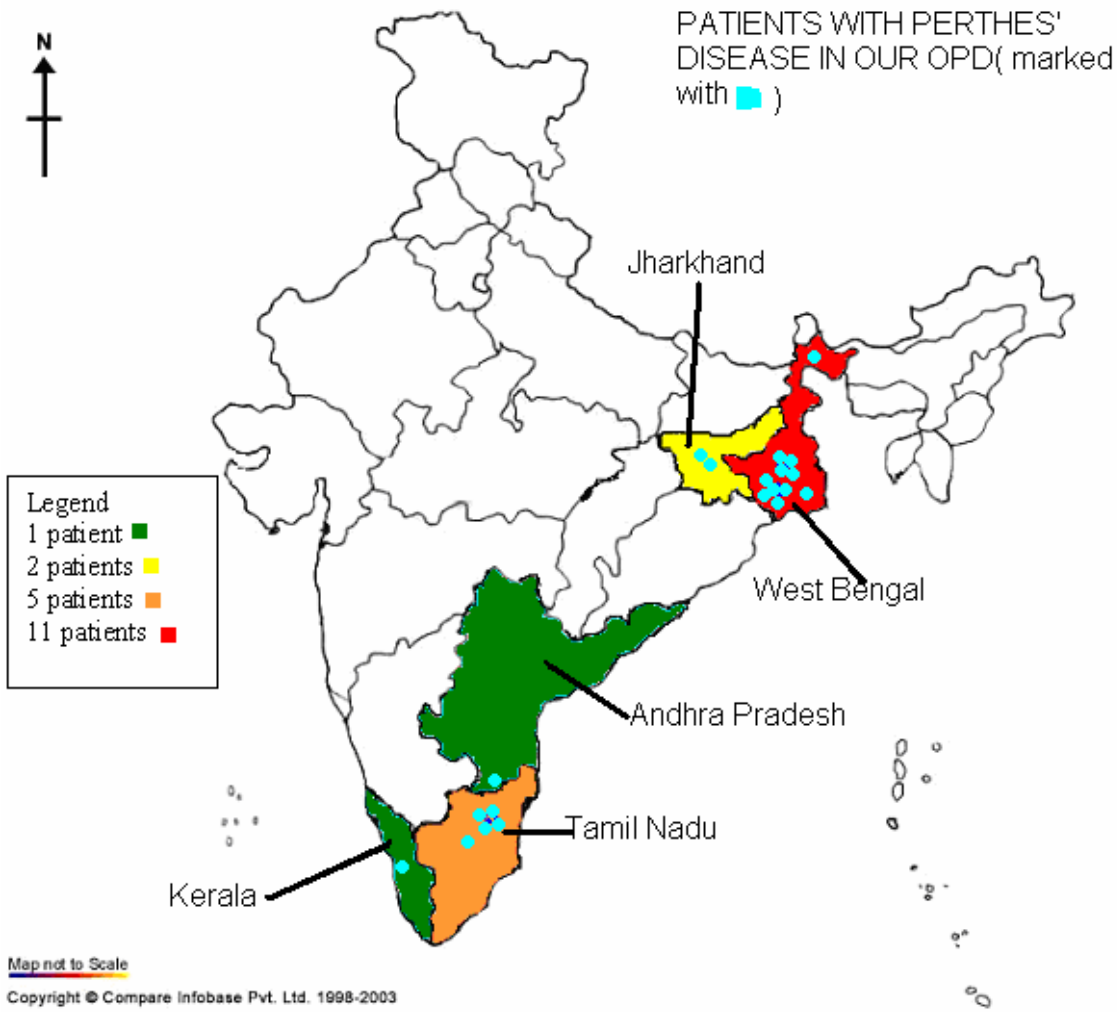


Figure 14. Distribution of cases in India

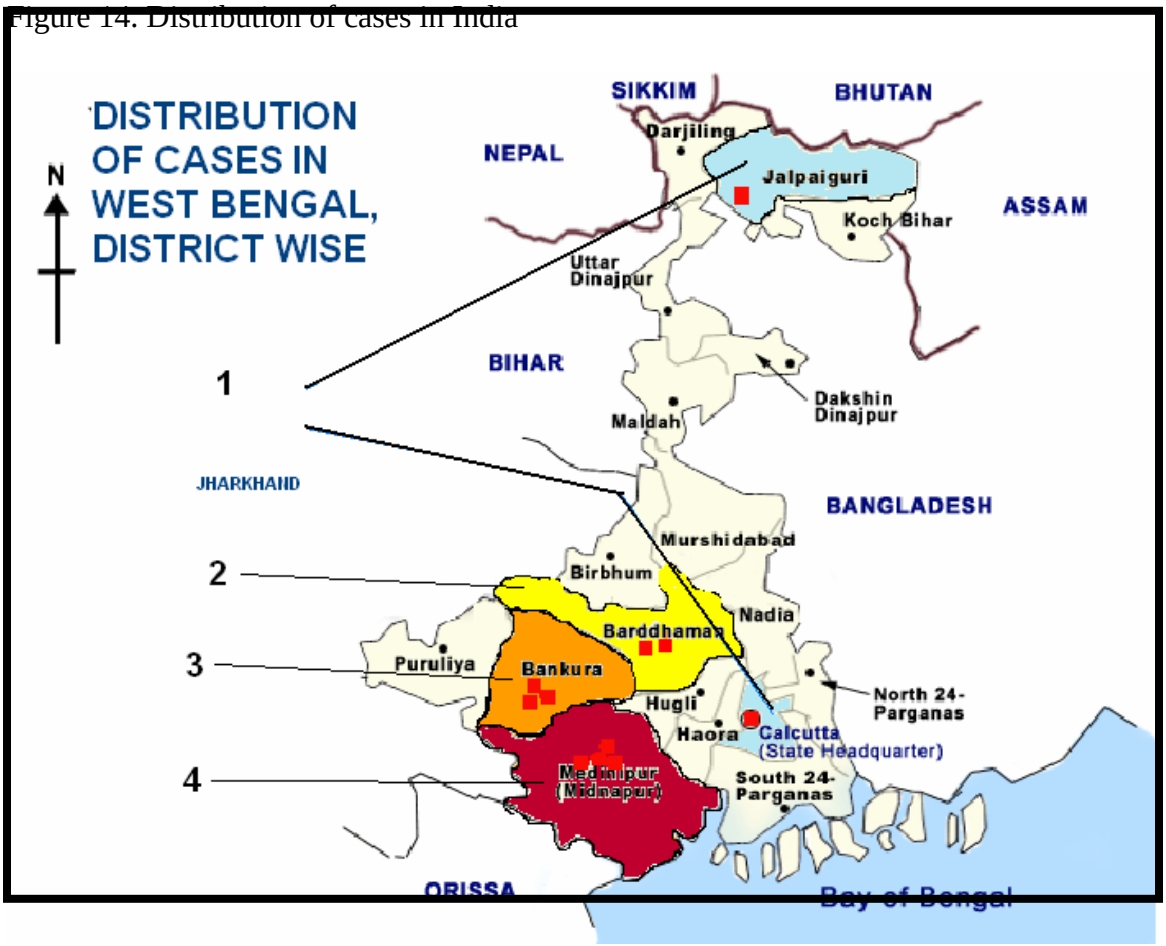


Figure 14. Distribution of cases in West Bengal. District wise

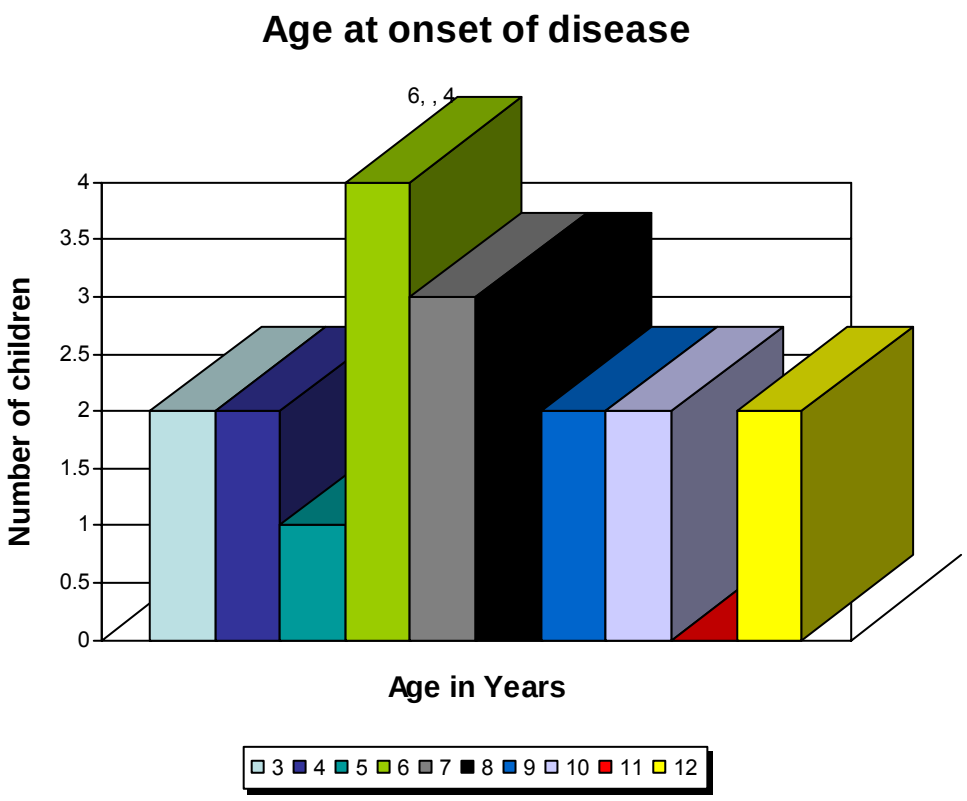


Figure 15. Age distribution of our patients.

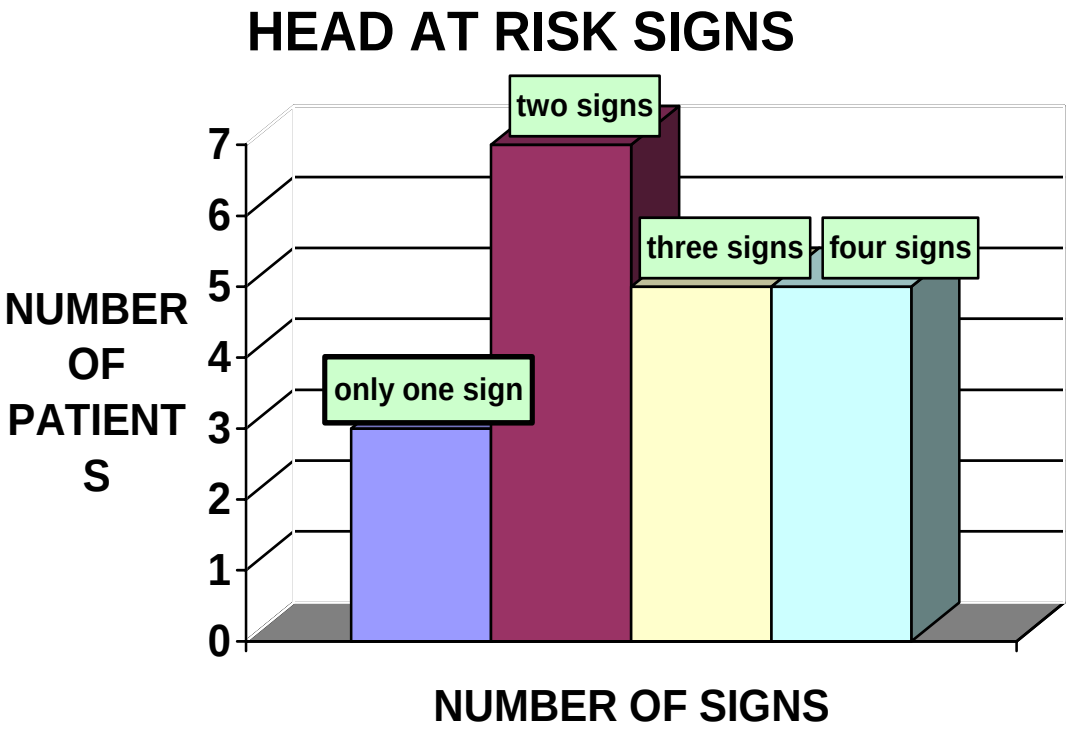


Figure 16: Head at risk signs frequency among our patients.

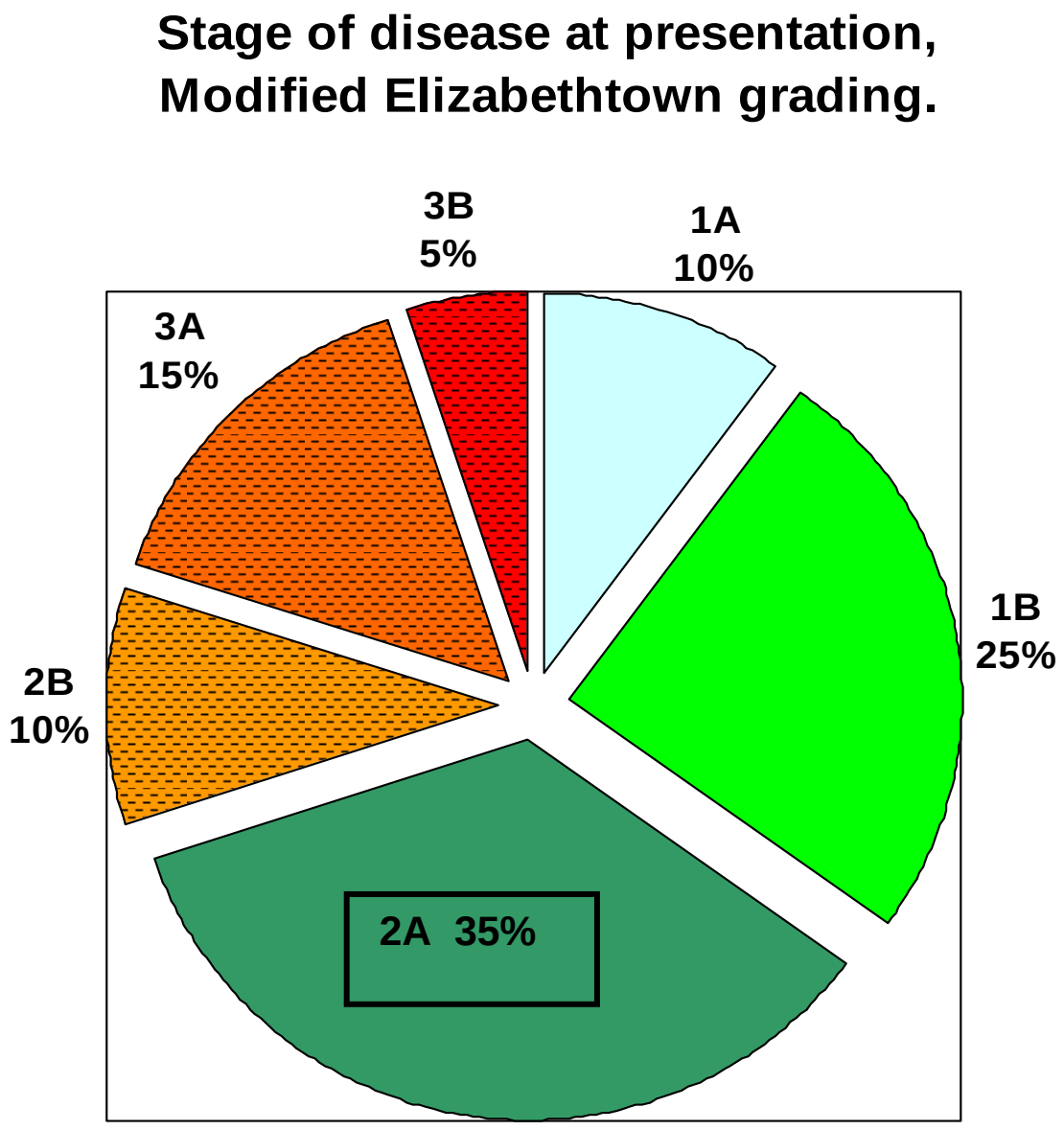


Figure 17: Stage of presentation of our patients in the OD.(Modified Eliabethtown grading)

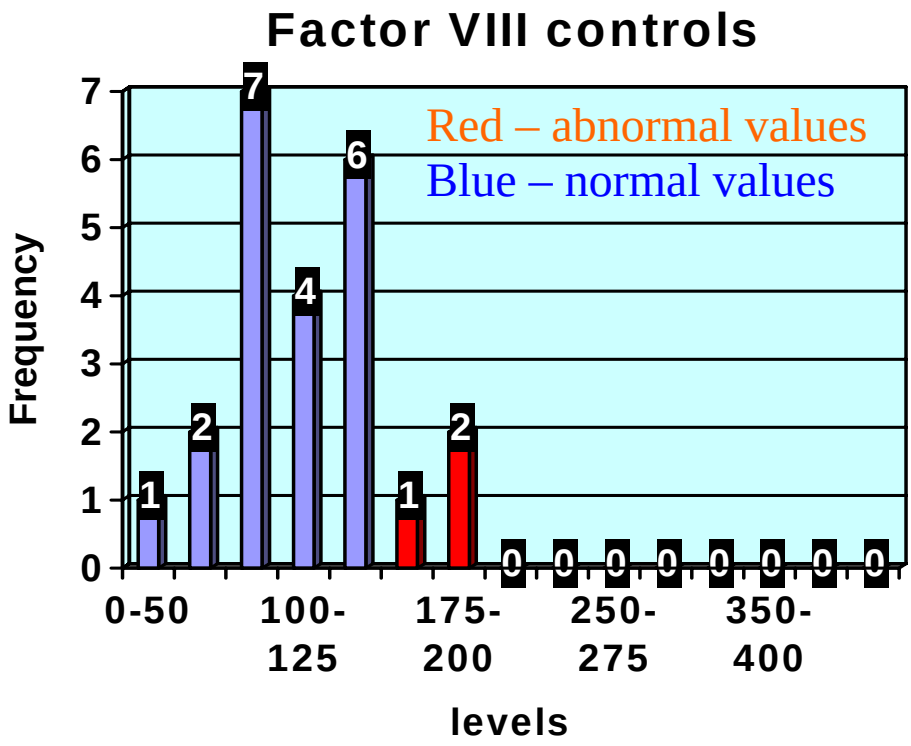


Figure 17: Distribution of abnormal values of FVIII in controls

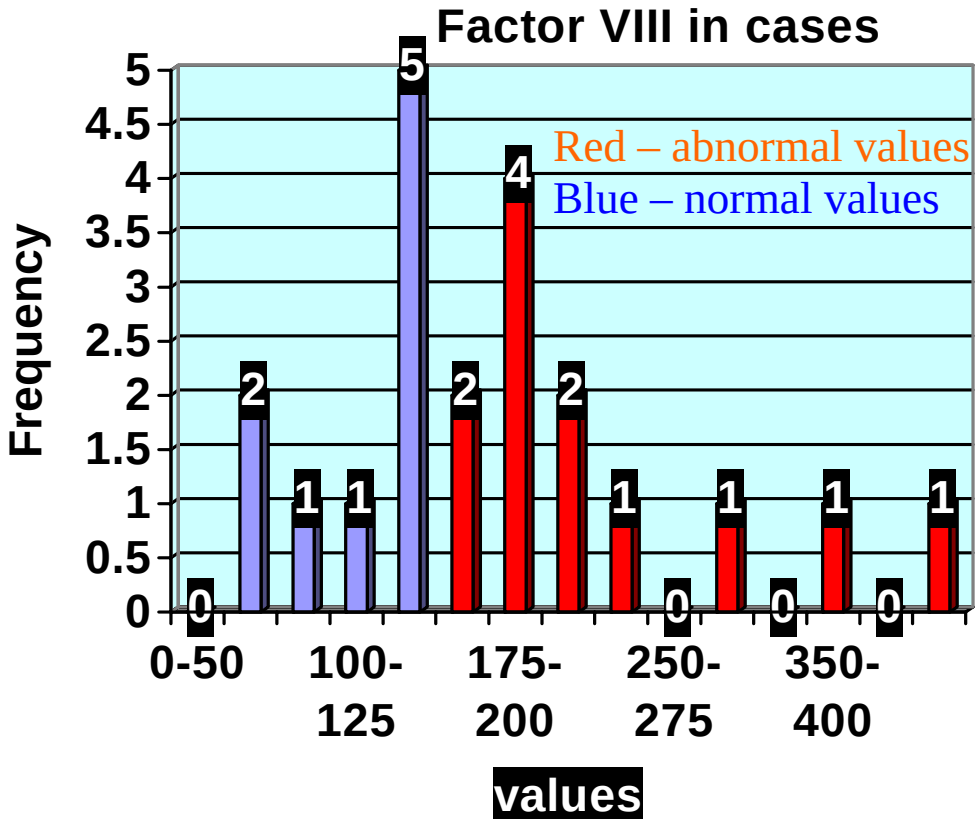


Figure 18: Distribution of abnormal values of FVIII in patients.

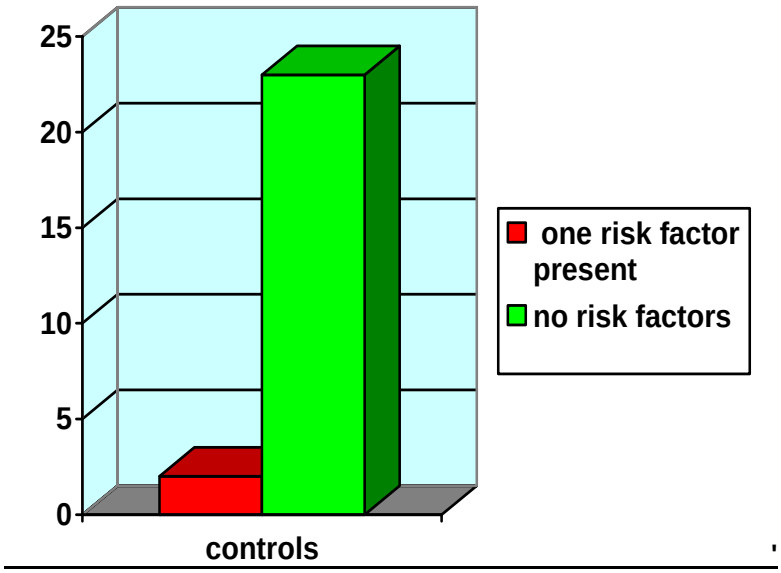


Figure 19: Number of controls who had any risk factor for thrombosis.

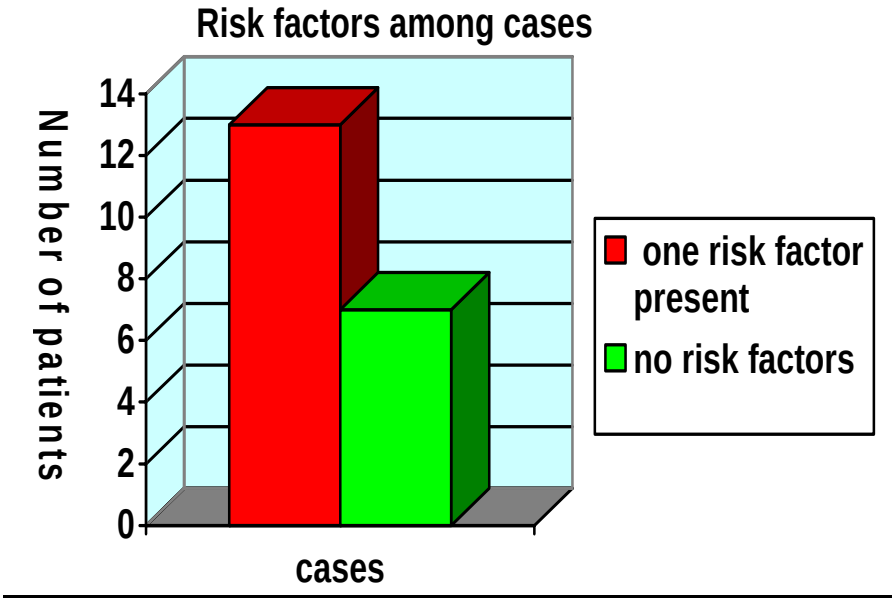


Figure 20: Number of patients who had any risk factor for thrombosis.

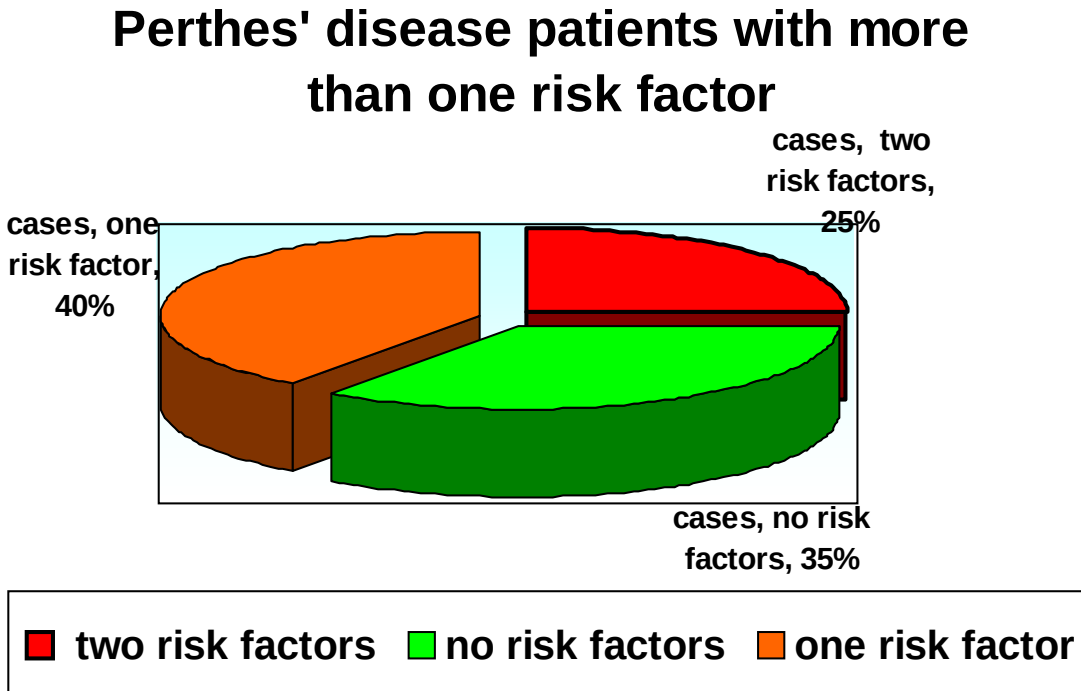


Fig 21: Distribution of risk factors among patients single and more than one. -65% of all patients had atleast one risk factor

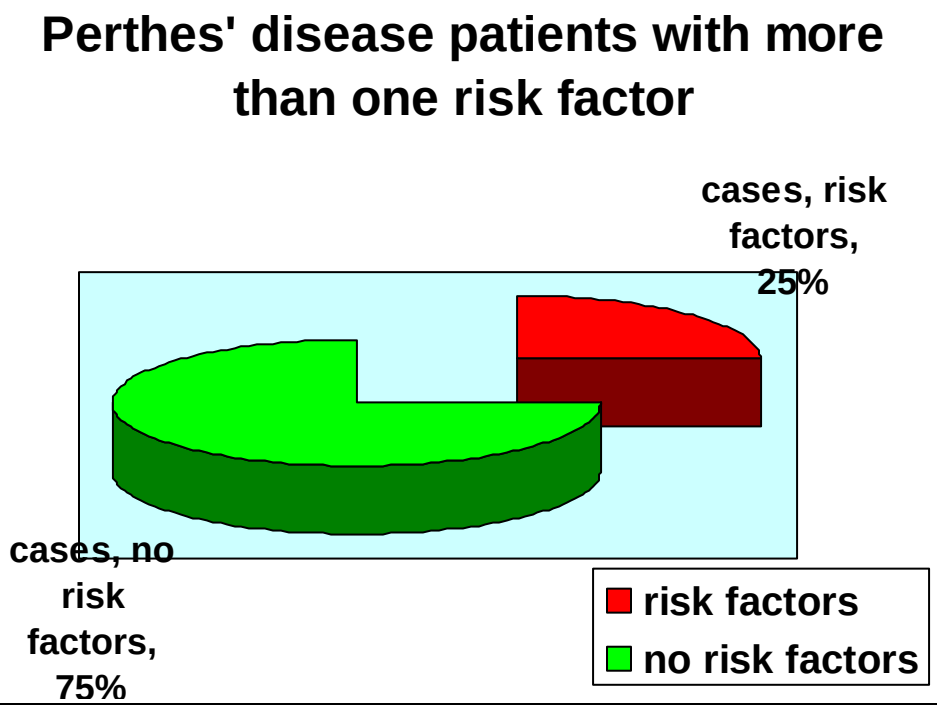


Fig 22: Distribution of risk factors among patients more than one risk factor. -25% of all patients had more than 1 risk factor

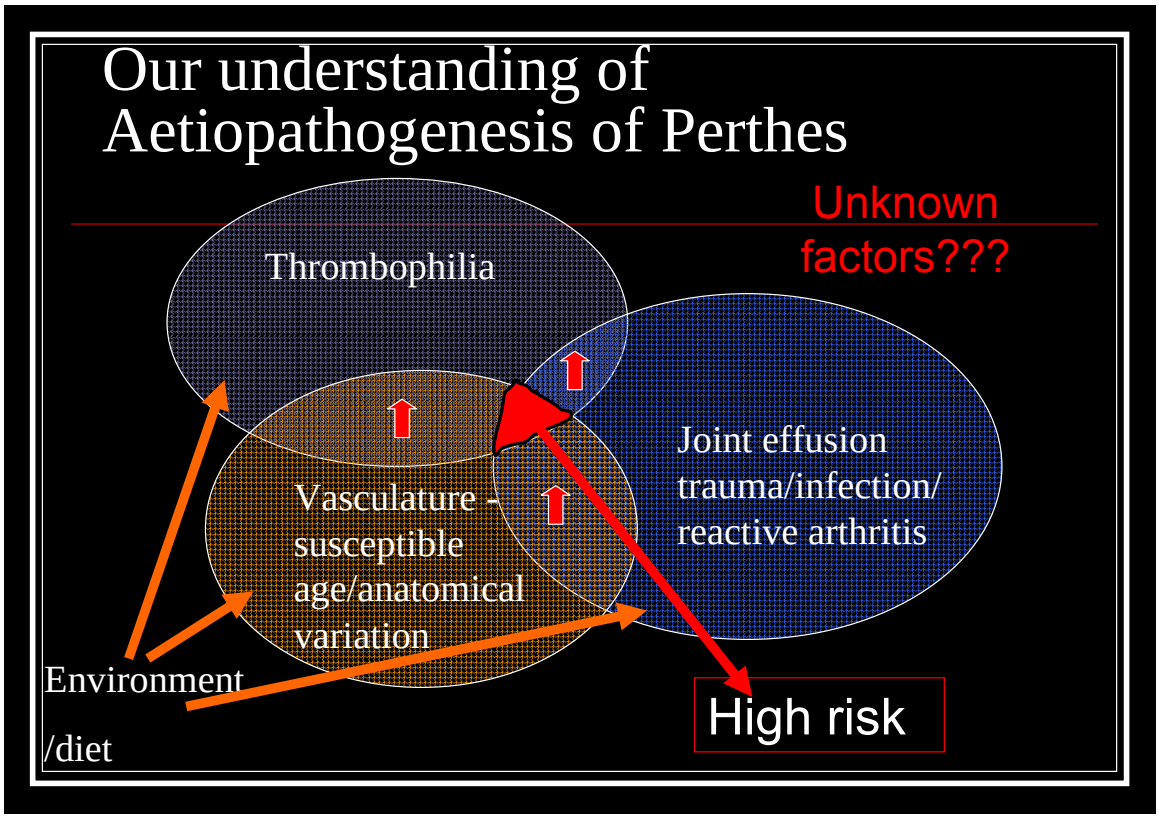


Figure 23. Our under standing of the Perthes disease etiology

ID	number	name	ospitalnumber		SEX	HEIGHT	SITTING HEIGHT	SUBISCHIAL HEIGHT
15	15	ABHISHEK ROY	803865C		M	108	63	48
21	21	ADDYAY ROY	845348C		M	128.5	67.5	61
13	13	HARUN MALIK	784022C		M	151	77	74
6	6	LALTOO GOPE	587398C		M	118	62	56
4	4	MANAS SINHA	649140C		M	125.5	66.5	69
2	2	NASIM ANSARI	760866C		M	114	61.00	53
19	19	NAVEEN	386108C		M	127	67	60
7	7	NISHA KUMARI	749497C		F	112	59.5	52.5
1	1	NOOR SHIKIN	654350C		F	100.5	61	39
9	9	PRAVEEN KUMAR	656910C		M	130.5	64.5	66
20	20	RAHUL	584496B		M	150.5	74	76
8	8	RAMYA	656167C		F	112	57	55
0	0	RANJAN	296950C		M	165.5	86.00	79.5
10	10	RANJAN KAR	652145C		M	128.5	67.5	61
11	11	ROHIT KUMAR	757643C		M	127.5	68.5	59
12	12	SALEHA BEGUM'S BABY	884082B		M	103	52.5	50.5
17	17	SHANKAR KUITY	540553C		M	138	68	70
5	5	SUBHANKAR DEY	609221C		M	133	69.5	64.5
22	22	SUDHAN	830789C		M	114	60	54
25	25	UJJAL GHOSH	793386C		M	124.5	60	64.5
3	3	KANNIYAMMAL	479526C		F	124.5	69.5	55

HEAD CIRCUM	UPPER LIMB LENGTH	ARM LENGTH	FORARM AND HAND	LL LENGTH	TIBIA LENGTH	FOOT LENGTH
50.5	43.5	18	29	54	22.5	16
51.5	55	23	35	66.5	29.5	20.5
51	68	27.5	0	79	34	22
48.5	53	21.5	32	61.5	25.5	17
51	56	22	34	67	30	18.7
51.5	41	19.5	30	58	24.5	17
52	57.5	23	36.5	65	28.5	20
48.5	46	19	30	58	25	16.6
50	44	17	20	52	22	16
51	56.5	25.5	36.5	69	29	19
52	66	28.00	40	83	37.5	21.4
47.5	48	27	30	58	25	15.2
55	76.5	31.5	45.4	92.5	41.5	25.1
53	56.5	24.5	35	66	29	17.5
51	53	24	36.5	68	29	19
48.5	43.5	18	27.5	50.5	22	17.5
55	61	26.5	38	74.5	32	21.5
51.2	60	24.5	38	68	29.6	19.2
53.5	48.5	20.5	31.5	58.5	25	17.5
51	52.5	22.5	34	63	28	18.5
47.5	51.5	19.5	28	62	26	20

BIACROMIAL DIST	BI ILIAC DIST	WT	protein c	protein s to	protein s fr	ANTI THROMBIN 3	SICKLE CE	PNH	Fib	F VIII	APCR
26	18	19	86	56.7	62.3	124.6	NEG	NEG	281.1	573.7	3.04
27.5	18	26.5	99.2	88.7	70.3	104.3	NEG	NEG	258	389	3.03
34	25	23.5	64.7	86.1	88.8	116.6	NEG	NEG	278	137.3	2.14
28	21	20	116.7	68.6	127.9	130.4	NEG	NEG	267	208	3.51
29	20.5	24	121.1	85	124.5	142	NEG	NEG	297	235	3.27
28	18.5	18.5	94.3	84.1	77.5	117.4	NEG	NEG	265.9	67.5	3.07
30.5	20	23.5	80.5	102	78.1	96.1	NEG	NEG	257	275.4	3.61
25.5	18.5	15.5	92.6	81	66.6	137.4	NEG	NEG	242	138.2	2.74
25	20	25	96.9	81.7	114.3	113.2	NEG	NEG	209	128	3.26
33.5	28.5	31	93.2	92.6	89.1	19.9	NEG	NEG	303	70.6	2.33
32	20	31	79.7	86.2	77.7	123.3	NEG	NEG	290	196	3.44
23.5	17.5	15	60.4	67.3	60.8	108.8	NEG	NEG	346	140.1	3.71
37.5	23.5	47.5	89.4	79	46.5	153.3	NEG	NEG	299	199	2.83
30.5	19.5	24.5	85.6	94.2	81.4	125.9	NEG	NEG	213	108.3	3.04
33	28	35	86.7	134.4	103.1	136.9	NEG	NEG	288.9	136.4	2.47
27	16.5	38	91	83	103.3	124.8	NEG	NEG	256	195.5	2.04
31.5	23	28.5	106.7	123.3	92.8	111.2	NEG	NEG	205	166.3	3.15
31.5	20	25	123.4	107.5	187.7	125.5	NEG	NEG	349.6	96.7	2.62
26	18.5	23	100.4	85.5	69	120.7	NEG	NEG	234	180	2.8
27	21.5	23.5	77.3	99.7	51.7	134.3	NEG	NEG	211.1	163	3.05
26	19.5	25	0	0	0	0	0	0	0	0	0

DRVVT	FACTOR V	FACTOR II	MTHFR 67	age	sex	age at blood testing	stage modi	herrings cla	salterthom	caterall gra	gage	lat extrusio	horizontal p
NEG	normal	normal	normal	4	M	5	1B	C	2	IV	YES	YES	YES
NEG	normal	normal	HETEROZ	8	M	8,4/12	1B	B	NOT DONE	IV	YES	YES	YES
NEG	normal	normal	normal	12	M	13	2B	C	2	IV	YES	YES	YES
POS MILD	normal	normal	normal	6,6/12(7)	M	8	1B	C	NOT DONE	IV	YES	NO	YES
NEG	normal	normal	normal	8,4/12,9	M	10	2A	C	2	IV	YES	YES	YES
NEG	normal	normal	normal	6	M	6,6/12	2A	C	2	III	YES	NO	NO
NEG	normal	normal	normal	6	M	9	1B	C	2	IV	NO	YES	YES
NEG	normal	normal	normal	9	F	9,6/12	2A	C	NOT DONE	IV	NO	YES	YES
NEG	normal	normal	normal	4,6/12	F	5	2A	B-C	NOT DONE	IV	NO	NO	NO
NEG	normal	normal	HOMOZYG	8,8/12	M	9	2A	B-C	2	III	YES	YES	NO
NEG	normal	normal	normal	7	M	11	1A	B-C	NOT DONE	III	YES	NO	NO
NEG	normal	normal	HETEROZ	8	F	10	2A	B	NOT DONE	IV	NO	YES	YES
NEG	normal	normal	normal	12	M	15	1A	A-B	NOT DONE	IV	YES	NO	NO
NEG	normal	normal	normal	6(9)	M	10	3A	B	NOT DONE	IV	NO	NO	YES
NEG	normal	normal	normal	7	M	10	3A	C	NOT DONE	II	YES	YES	YES
POS MILD	normal	normal	normal	5	M	6	1B	A	NOT	IV	NO	NO	NO
NEG	normal	normal	normal	104/12,11	M	13	2B	C	NOT DONE	IV	YES	YES	YES
NEG	normal	normal	normal	9	M	10	3A	C	NOT DONE	IV	NO	YES	NO
POS MILD	normal	normal	normal	5,6/12	M	5,1/2	2A	C	NOT DONE	IV	YES	NO	NO
NEG	normal	normal	normal	3,8/12,7	M	10	3B	C	NOT DONE	IV	NO	YES	YES
0	0	0	0	10	F	11	2A	C	2	III	NO	YES	YES

cysts meta	METAPHY	TRAUMA	FEVER	lat calcification		Controls	protein c	protein s to	protein s fr	ANTI THR	SICKLE CE	PNH	Fib
YES	NO	NO	YES	NO	1	Control	64.8		46.7	143.8	neg	neg	
YES		NO	NO	NO	2	Control	50.7		54.3	129.2	neg	neg	
YES	?	NO	NO	YES	3	Control	129.7		60	148.8	neg	neg	
YES		YES		NO	4	Control	126.9		68.9	122.2	neg	neg	
YES		NO		NO	5	Control	124		111.7	127.5	neg	neg	
YES		NO	NO	NO	6	Control	101.5		81.4	156.3	neg	neg	
NO		YES	NO	YES	7	Control	157.9		92.7	141.9	neg	neg	
YES	NO	NO		YES	8	Control	132.5		104.7	124.4	neg	neg	
YES		NO	NO	NO	9	Control	104.3		125.9	130.8	neg	neg	
YES	NO	NO	NO	YES	10	Control	121.2		63.7	121.2	neg	neg	
YES	NO	NO		NO	11	Control	124		100.1	122.4	neg	neg	
YES	NO	NO	YES	NO	12	Control	64.8		162.4	149.8	neg	neg	
YES		NO	NO	NO	13	Control	84.6		198.9	129.9	neg	neg	
YES		NO	NO	YES	14	Control	76.1		112.4	129.6	neg	neg	
NO	NO	NO	NO	YES	15	Control	56.4		64.2	126	neg	neg	
YES	NO			NO	16	Control	70.5		77.9	124.4	neg	neg	
YES	YES	NO		YES	17	Control	90.2		71	127.7	neg	neg	
YES		YES	NO	YES	18	Control	64.8		66.1	130.8	neg	neg	
YES	?	NO	NO	NO	19	Control	98.7		198.9	108	neg	neg	
YES		NO	YES	YES	20	Control	667.6		58.6	151.7	neg	neg	
YES	YES			NO	21	Control	101.5		142.8	140.4	neg	neg	
					22	Control	115.6		73.3	118.6	neg	neg	
					23	Control	98.7		125.4	121	neg	neg	

F VIII	APCR	DRVVT	FACTOR V	FACTOR II	MTHFR 677	
107.4	2.06	neg	neg	neg	neg	
170.8	2.16	neg	neg	neg	neg	
136.4	2.17	neg	neg	neg	neg	
133.4	2.35	neg	neg	neg	neg	
98.6	2.12	neg	neg	neg	neg	
89.2	2.02	neg	neg	neg	neg	
76.4	1.66	neg	neg	neg	neg	
98.6	2.15	neg	neg	neg	neg	
113.4	2.13	neg	neg	neg	neg	
69.6	2.2	neg	neg	neg	neg	
87.8	2.15	neg	neg	neg	neg	
122.4	1.98	neg	neg	neg	neg	
144	2.18	neg	neg	neg	neg	
176	2.03	neg	neg	neg	neg	
120.6	2.25	neg	neg	neg	neg	
134.4	1.77	neg	neg	neg	neg	
49.6	1.68	neg	neg	neg	neg	
125.4	1.76	neg	neg	neg	neg	
148.6	1.62	neg	neg	neg	neg	
66.4	1.66	neg	neg	neg	neg	
84.4	1.7	neg	neg	neg	neg	
187.4	1.69	neg	neg	neg	neg	
85.8	1.68	neg	neg	neg	neg	