PREVALENCE OF FACTOR VIII INHIBITORS IN HAEMOPHILIA A PATIENTS WHO RECEIVED FACTOR VIII THERAPY.

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LIST OF ABBREVIATIONS

FVIII	_	Factor VIII
pdFVIII	_	Plasma-derived Factor VIII
BU	_	Bethesda Units
rFVIII	_	Recombinant Factor VIII
BDD-rFVIII	_	B domain-depleted rFVIII
IL10	_	Interleukin-10
TNF α	_	Tumor Necrosis factor -α
CTLA4	_	Cytotoxic T-lymphocyte antigen-4
MHC	_	Major Histocompatability Complex
CANAL	_	Concerted Action on Neutralizing Antibodies in Severe Hemophilia A
UKHCDO	_	United Kingdom Hemophilia Centre Doctor's Organization
SIPPET	_	Survey of Inhibitors in Plasma-Product Exposed Toddlers
PUPS	_	Previously untreated patients
FFP	_	Fresh Frozen Plasma
FEIBA	_	Factor VIII Inhibitor Bypassing Agents

INTRODUCTION

Haemophilia A is a common, X-linked, inherited congenital bleeding disorder. It is caused by dysfunctional or deficient production of coagulation Factor VIII (FVIII). Factor 8 gene is located in the long arm of X chromosomes. It is one of the largest gene with the molecular weight of 260 kDa . Since it is a X - linked recessive disorder ,it occurs in males and the females are carriers.[2] As early as in the 2nd century, hemophilia was recognised and the cardinal features of hemophilia were described.[3] Since 1937,it was found that the plasma component Anti hemophilic factor, named as Factor VIII was deficient in hemophilia patients .Factor VIII plays a critical role in the propagation phase of coagulation. [4]

The incidence of haemophilia A is 1 in 5000 male live births. Estimated of Haemophilia A is one in 10,000 births approximately.[5] World Federation of Hemophilia Survey indicate the number of hemophilia in the world wide is approximately 4,00,000 .[5] Hemophilia A prevalence is "the total number of reported or identified cases of Haemophilia A in the population at a given time divided by the total number of males in that population".[6] In India ,the number of expected Patients with hemophilia would be approximately 120000.According to World Federation of Hemophilia Global survey 2010 only 13993 patients were registered. This indicates the gross under diagnosis, early deaths and lack of awareness to access treatment facilities.[80] The prevalence of Haemophilia A for high income countries was 12.8 per 100 000

males and 6.6 per 100 000 males in the rest of the world.[5] Haemophilia A is more common than Haemophilia B ,representing 80-85% of the total haemophilia population.[7]

Clinical manifestations of Haemophilia A are easy bruising, spontaneous bleeding into the joints , muscles and soft tissues, excessive bleeding after trauma.[5] Classification of hemophilia is based on plasma procoagulant levels with persons <1% factor defined as severe,1-5% as moderate,>5% and less than 30% is mild.[9] In mild haemophilia ,the patient bleed excessively only after trauma or surgery. Patients with Severe haemophilia A have spontaneous bleeding or excessive bleeding after minor trauma, particularly into joint muscles.[9]

Treatment of Haemophilia A patients relies on administration of exogenous Factor VIII either in the form of Fresh Frozen plasma/ cryoprecipitate ,plasma derived Factor VIII or recombinant Factor VIII.[9] The current standard of care for Haemophilia A patients is either on demand or prophylactic.[10] Plasma derived Factor VIII concentrates used for supplementation therapy in the 1980s were frequently contaminated with human deficiency virus. Hepatitis B and C viruses causing mortality in a large hemophilic population .[11] The problems related with transfusion transmitted infections can be prevented by the use of recombinant Factor VIII.

Development of antibodies against the exogenous Factor VIII is the major potential complication of the treatment of Haemophilia A. This antibodies are known as inhibitors .[12] These Factor VIII inhibitors are IgG immunoglobulins.

Inhibitors are classified according to their levels in plasma as high titre, those with ≥5 Bethesda units/ml and low titre inhibitors <5 Bethesda units/ml. Some patients develop transient inhibitors ,these are low titre inhibitors that never exceed a titre of 5 BU/ml and disappear spontaneously with time.[8] One Bethesda unit is defined as the inhibitory titre needed to inactivate 50 percent of the Factor VIII present in normal plasma within 2 hour incubation period at 37°C.[13]

Over the years, numerous risk factors both treatment related and patient related have been identified. The development of inhibitors related to the patient characteristics including severity of the disease, ethnicity, and mutation involved in F8 gene, family history of inhibitors and the treatment related risk factors like intensity of Factor VIII exposure, early exposure to Factor VIII and product type. According to the severity of disease, the persons with severe haemophilia A are at greatest risk for inhibitor development .[14] Risk of inhibitor formation has varied between 20-30% in patients with severe haemophilia A and 3-13% in those with mild or moderate haemophilia A.[15]

Presence of inhibitor is a challenging problem in the treatment of Haemophilia A to control bleeding manifestation, maintain the haemostasis and overall quality of life.[16] Inhibitor assays are performed in haemophilia A patients as a screening procedure when the presence of inhibitor is suspected in the case of abnormal bleeding in spite of taking Factor VIII or poor response to Factor VIII replacement therapy. Among the haemophilia A patients facing the challenges, the development of inhibitors is the most feared one.

With this background our study aims to describe the prevalence of inhibitors and the association between the risk factor and inhibitor development.

AIMS & OBJECTIVES

Aim :

Aim of our study is to find out the prevalence of Factor VIII inhibitors in haemophilia A patients who received plasma derived Factor VIII therapy.

OBJECTIVES :

- To estimate the Factor VIII levels and classify the haemophilia A
- To Identify the risk factors for inhibitor development

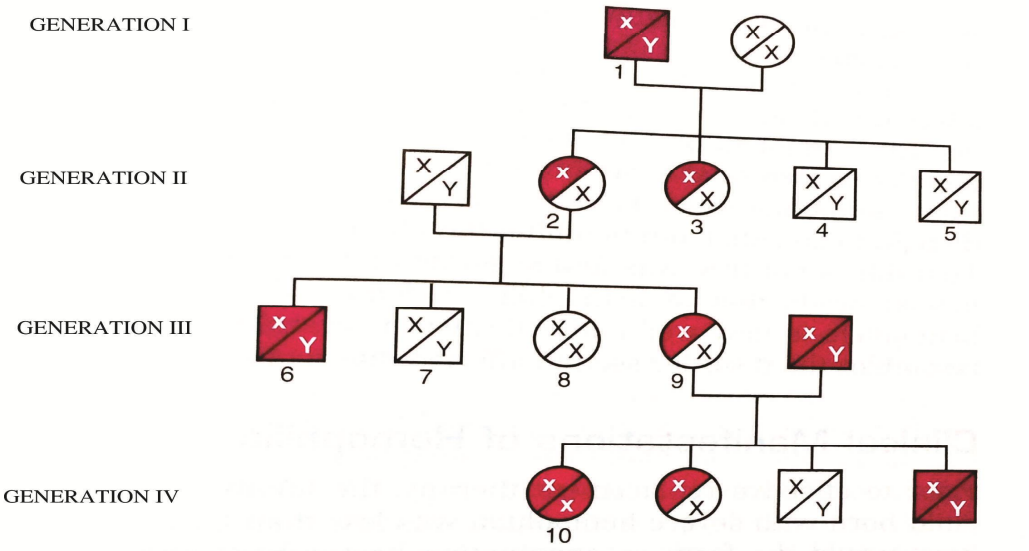
REVIEW OF LITERATURE

Haemophilia A is the most common severe inherited bleeding disorder.[4] The hereditary and sex linked nature of the disease was recognized since ancient times. As early as in the 2nd century the haemophilia was recognized in the Jewish writings. Rabbinic references showed the characteristic feature of the disease was the fatal bleeding after minor injury in brothers or in maternally related boy cousins.[3] In the 10th century , khalaf ibn abbas described the males who bled to death after minor injury.[3] The Arabic physician Albucasis who lived in the 12th century described males in a family died after a trivial injury.[3,17] In the 18th century Dr John Conrad otto a physician from philadelphia described the cardinal features of haemophilia ,the symptoms were shown only by males, the unaffected females transmit the disease to a proportion of their sons.[17,3] In 1828 Hopff who was the student at the university of zurich who is the first one use the term "HAEMOPHILIA". In 1890, the involvement of joint is the characteristic symptom of haemophilia was described by konig. Haemophilia has been called the ROYAL DISEASE. Queen Victoria of ENGLAND (1837-1907) was a carrier of the haemophilia gene and subsequently passed the disease on to several royal families.[3]The bleeding tendency of haemophilia was due to fragility of blood vessels.[3] In 1930 ,the deficiency of platelets were thought to be the main cause of bleeding.[25]Since 1937 Peter J studied the deficiency of plasma component had some association with Haemophilia, then Patek and Taylor described about the "Anti Haemophilic factor" was derived from the plasma of normal individual which one corrects the clotting defect of haemophilia. After that according to

the recent nomenclature that anti haemophilic factor was known as Factor VIII.[18]

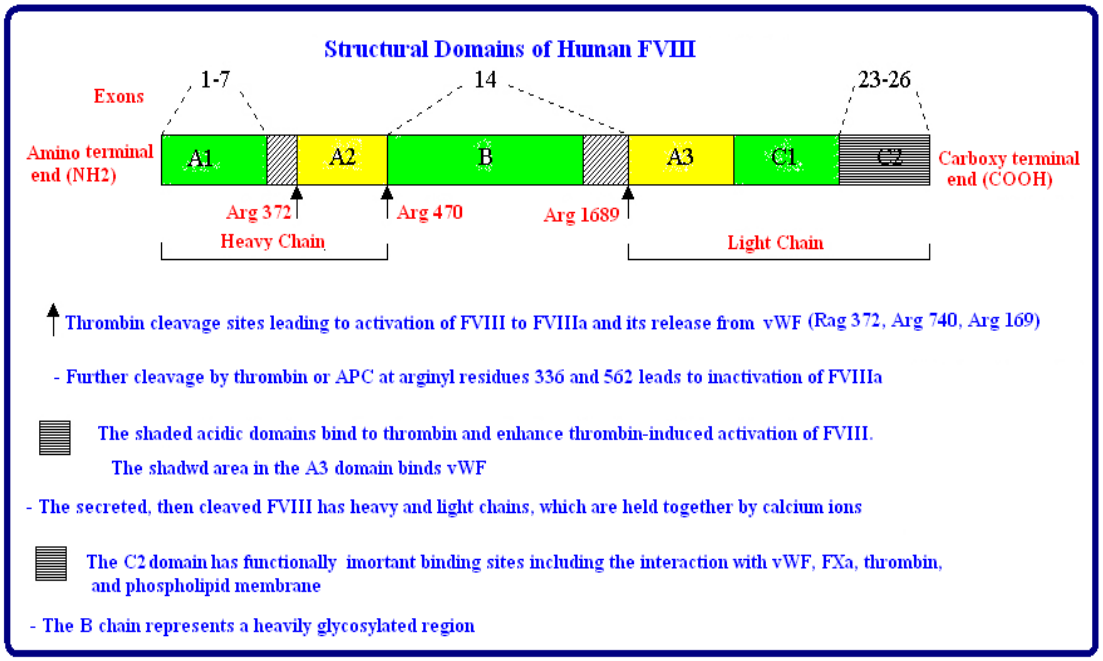
In the 19th century Quick described the laboratory definition included the concepts of long blood clotting time, normal prothrombin time, normal platelets count but an obvious prolongation of the plasma clotting time after spinning down the platelets. After laboratory work up , they found ,the reduction of Factor VIII is the central feature of haemophilia.[3] Haemophilia A is the inherited deficiency of blood coagulation factor leads to lifelong bleeding disorder ,the factors most commonly found deficient are factor VIII and factor IX these genes are located in the X chromosomes.[1] Haemophilia A is an X-linked congenital bleeding disorder resulting from a deficiency of Factor VIII.[20] Since these disorders are X- linked they usually occurs in males.[2] The world wide incidence of haemophilia A is approximately 1 case per 5000 male individuals.[9] 30% of cases a spontaneous mutation and there is no family history of haemophilia.[2,4] Female carriers are expected to have a plasma concentration of factor VIII corresponding to half the concentration found in healthy individuals, which is generally sufficient for normal haemostasis.[4] 50% chance of carrier mother transmit the defective X -linked gene to the male or female child. All female offspring are obligatory carriers born to carrier father.[21] Even though the severe cases of hemophilia is rare in female, genetic mechanism can result in clinical manifestation of haemophilia A in females .The genetic mechanisms involved [4] are Inheritance of homozygous F8 mutation, Skewed inactivation of the X chromosome , X/O karyotype: Turner syndrome and X/ autosome translocation.10% higher incidence seen in the consequence of consanguineous marriages.[7]

FIGURE.I. PEDIGREE CHART



The Inheritance of hemophilia A and Hemophilia B. The pedigree is hypothetical Squares indicate male; circles indicate female; fully shaded squares or circles indicate affected members: half – shaded circles indicate carriers.

FIGURE .2.THE STRUCTURE OF FACTOR VIII PROTEIN



STRUCTURE OF FACTOR VIII :

FVIII gene is located in the long arm of X chromosome. It is one of the largest genes known with the molecular weight of 260 kDa.[4] It spans over 180 kb. Analysis of the primary structure showed the presence of discrete domain structureA1-a1-A2-a2-B-a3-A3-C1-C2.The A domain display approximately 30% homology to each other, C domains are structurally related to factor V, the B domain is unique ,no significant homology with any other protein. The Factor VIII gene comprises 26 exons which encode polypeptide chain of 2351 amino acids. This includes signal peptide of 19 and a mature protein 2332 amino acids. Rough endoplasmic reticulum and Golgi apparatus of hepatocytes, are the primary source of FVIII in liver but not in sinusoidal cells and endothelial cells.[18]

SECRETION AND CIRCULATION OF FACTOR VIII :

The FVIII is secreted into the circulation, after synthesis in the hepatocytes, in the circulation it forms non covalent bond with vWF .The plasma concentration of FVIII and vWF is 100 to 200 ng /ml and 10µg/ml respectively. vWF binds to the A3 and C2 regions of FVIII through sequence in the D´/D3 region of the mature vWF monomer. In the plasma vWF protects FVIII from proteolysis by activated protein C. Without this interaction the plasma half life of FVIII is reduced and the plasma levels of FVIII are low.[4]

FIGURE.3.COAGULATION CASCADE CELL BASED MODEL

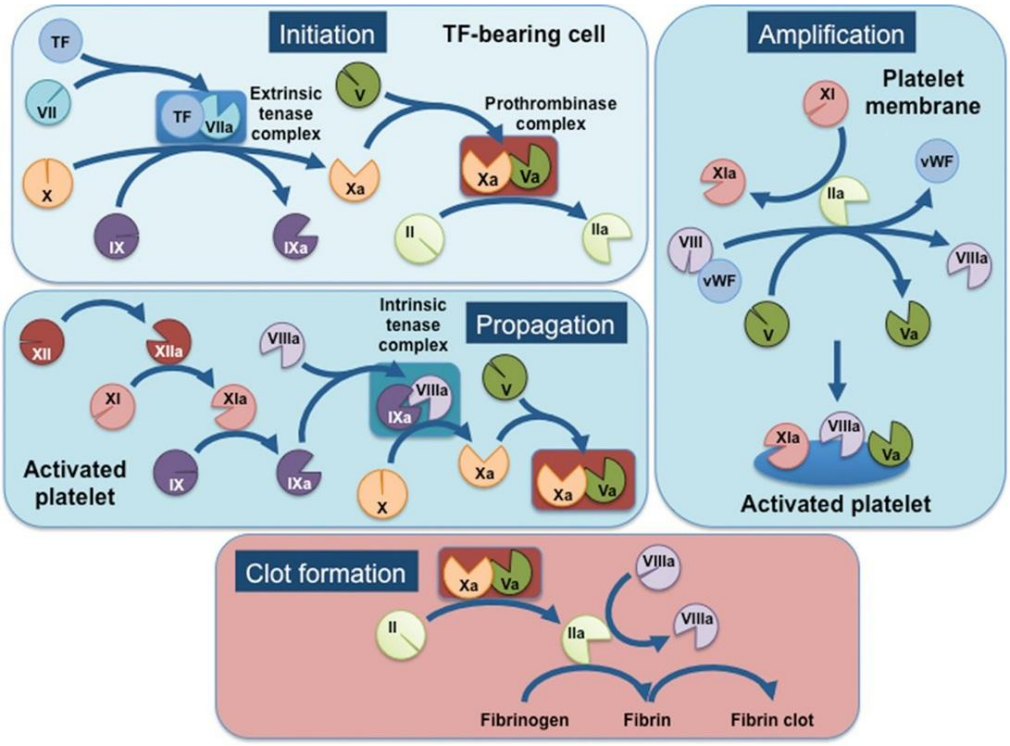


FIGURE.4.INTRINSIC TENASE COMPLEX FORMATION

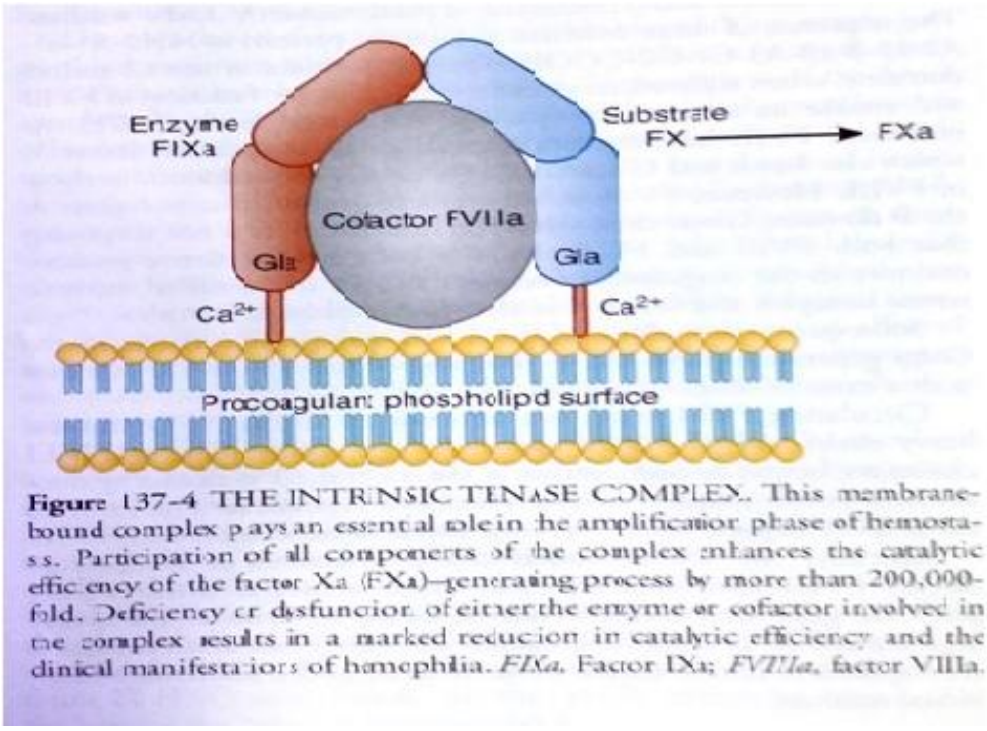


Figure 137–4 THE INTRINSIC TENASE COMPLEX. This membrane-bound complex plays an essential role in the amplification phase of hemostasis. Participation of all components of the complex enhances the catalytic efficiency of the factor Xa (FXa)–generating process by more than 200,000-fold. Deficiency or dysfunction of either the enzyme or cofactor involved in the complex results in a marked reduction in catalytic efficiency and the clinical manifestations of hemophilia. *FIXa*, Factor IXa; *FVIIIa*, factor VIIIa.

FVIII plays a critical role in the propagation phase of coagulation .Thrombin is the physiological activator of FVIII, which proteolytically cleaves the FVIII at three sites, Arg 372 at the NH2 terminus of the A2 domain, Arg 740 at the NH2 terminus of B domain and Arg 1689 at the NH2 terminus of the A3 domain. These cleavage results in the release of FVIII from vWF and the formation of non covalently associated activated FVIIIa. Activated FVIII forms essential cofactor activity in the intrinsic tenase complex , where FIXa is the serine protease and FX is the substrate. FVIIIa enhances the catalytic reaction about 200,000 fold. Severe deficiency profoundly reduces the rate of generation of FXa

FIGURE.5. FACTOR VIII PROTEIN ACTIVATION AND INACTIVATION

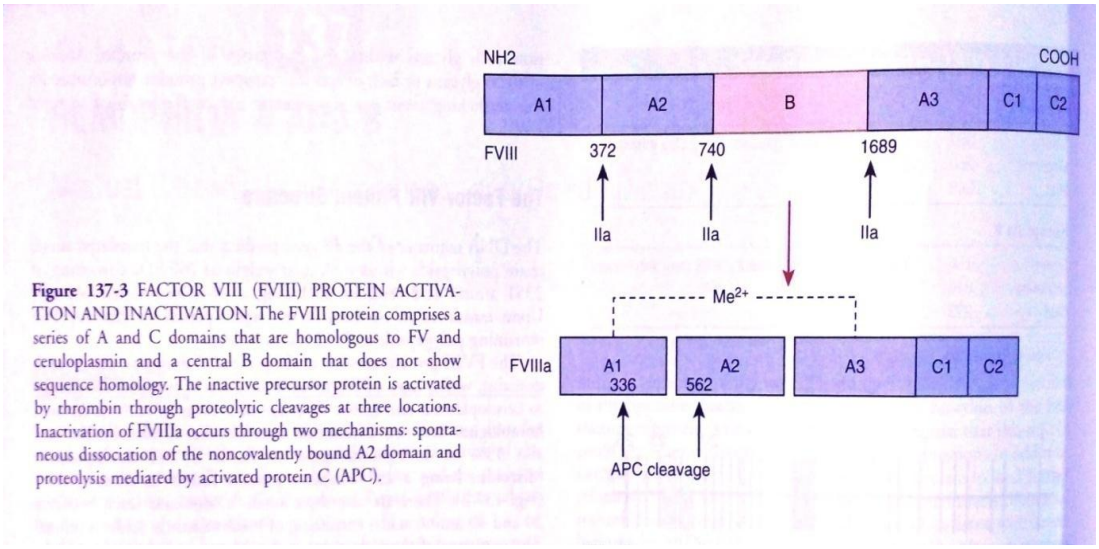


Figure 137-3 FACTOR VIII (FVIII) PROTEIN ACTIVATION AND INACTIVATION. The FVIII protein comprises a series of A and C domains that are homologous to FV and ceruloplasmin and a central B domain that does not show sequence homology. The inactive precursor protein is activated by thrombin through proteolytic cleavages at three locations. Inactivation of FVIIIa occurs through two mechanisms: spontaneous dissociation of the noncovalently bound A2 domain and proteolysis mediated by activated protein C (APC).

Two processes were involved in the inactivation of FVIIIa.

1. Spontaneous dissociation of the A2 domain
2. Activated protein C mediated proteolysis at Arg 562 in the FVIIIa heavy chain.

MOLECULAR DEFECTS IN HAEMOPHILIA A:

Impaired transcription, RNA processing or translation of FVIII gene are due to gross deletion or rearrangements of gene ,it may result in reduced synthesis of factor VIII or the release of dysfunctional FVIII .Single missense mutation cause defective secretion.[22] A defect located outside the FVIII gene also the cause of reduced levels of FVIII gene. 40-50% of the haemophilia A has an association with intron 22 inversion **.**40% of the cases are caused by an inversion involving a gene within intron 22 of the FVIII gene. Inversion in intron 1 has also been detected recently. Severe deficiencies associated with missense mutation in which impaired folding and or altered conformation of the mutant FVIII lead to both intra and extracellular instability which in turn causes severe factor deficiency in plasma. [19,23,22,24]

CLASSIFICATION OF HAEMOPHILIA A:

Classification of the severity of haemophilia has been based on either clinical bleeding symptoms or plasma procoagulant levels ,the later is widely used.[8] The amount of residual FVIII present determines the clinical variability of the disease.[22]Haemophilia A classified as mild (6%to 30%FVIII level) ,moderate (1 to 5%)and severe(<1%).[16] Haemophilia was classified based on clinical symptoms, because severe haemophiliacs may exhibit no spontaneous bleeding and appears to be clinically normal, conversely midland moderate haemophilia patient may have frequent spontaneous bleeds and appear to be

clinically severe.[8] Mild haemophiliacs bleed excessively only after trauma or surgery. Severe haemophilia have an average of 20 to 30 episodes of spontaneous or excessive bleeding after minor trauma.[16.]

CLINICAL FEATURES OF HAEMOPHILIA A:

Dysfunctional factor VIII disrupts the normal intrinsic coagulation cascade. based on factor VIII activity .[26] The bleeding tendency is determined by the baseline level of the deficient or defective clotting factor. Spontaneous bleeding is frequent in severe haemophiliacs.[4] In mild hemophilia ,spontaneous bleeding is infrequent but prolonged and excessive bleeding occur after trauma, invasive surgical or dental procedures.[4,26] Haemarthrosis is a classical clinical sign of severe hemophilia.[4] Common sites involved are ankle, knee, elbow. In addition to joint bleeding prolonged soft tissue and mucocutaneous bleed also common in hemophilia A. Epistaxis is not a common feature of haemophilia but it can occur.[4] Hematuria is common in haemophiliacs. Gastro intestinal bleeding occur in haemophilia A adult those who are using NSAIDs for haemophiliac arthropathy. Intra cranial haemorrhage is most common in both neonates and children with hemophilia .The incidence in newborn with severe hemophilia is 3,5% to 4%.incidence is highest in neonates associated with traumatic vaginal delivery.[4] Intra cranial hemorrhage in patients younger than 18 years and can be fatal.[27]

1950s and 1960s, haemophiliacs could be treated only with whole blood or fresh plasma, in severe haemophiliac patients, quantity of FVIII in fresh plasma is not enough to stop hemorrhage after surgery or trauma or in vital organs. In 1964 Judith pool discovered that cryoprecipitate from plasma contained large amounts of FVIII.[17] The lyophilised plasma concentrates of coagulation factors were available in 1970s and the widespread adoption of home replacement therapy led to the control of haemorrhage. For producing plasma derived Factor VIII concentrate the large size of donor pools were included, this was heightened the risk of viral contamination.[27] In 1980s the people who received the plasma products were infected with HIV and HCV.[3] As a consequences of this, the need for safe treatment became crucial for the haemophilia community.[27] The viral inactivation techniques been developed and implemented for the production of plasma derived FVIII concentrates, the new methods been adopted in for transfusion transmitted infection.[19] In 1984 the rapid progression in DNA technology, allowed the industrial production of recombinant FVIII.[17] In 1987 March, the recombinant FVIII was infused in a haemophilia patient.[28] rFVIII is derived through heterologous transfection rFVIII DNA plasmids into a non human mammalian cell line, either Chinese hamster ovary or baby hamster kidney cells. The expressed proteins are then secreted into a culture medium containing human or bovine protein for stabilisation. The recombinant proteins are then purified via various chromatographic techniques.[28] The improvement of protein purification techniques the addition of viral inactivation steps recombinant helped to reduce

the TTI.[17] Advancement in developing the recombinant FVIII ,the majority of patients in developed countries treated with recombinant products.[29] Several recombinant Factor VIII products are available ,the majority of them containing full length Factor VIII molecules stabilized either by human serum albumin or sucrose solution.[30] Another type is B domain depleted rFVIII approximately 38% of the primary cDNA could be removed without loss of procoagulant activity was demonstrated. No addition of plasma derived albumin was needed for stabilization of the final product. when compared to full length rFVIII BDD-rFVIII provided safe, well tolerated and effective treatment of haemophilia A.[28] However the TTI can be avoided by recombinant, the major complication of infusion of exogenous FVIII is the development of inhibitory allo antibodies against exogenous FVIII.

FACTOR VIII INHIBITORS :

INTRODUCTION :

Apart from TTI complication ,the inhibitor development is the most significant complication[20], it is a complex interaction between patient's immune system and genetic and environmental risk factors.[31] Inhibitors reduce the efficacy of haemostatic treatment and clearly cause additional morbidity.[32] "Prevalence indicates only proportion of patients with an inhibitor at an particular period of time".[33]

IMMUNOBIOLOGY OF FVIII INHIBITORS:

Synthesis of inhibitors depends on the activation of CD^{4+}(helper) T cells specific for FVIII .[34]

- Administered FVIII molecule is endocytosed by a antigen presenting cell(APC)
- Intra cellular proteolytic degradation ->generation of short component peptides
- Major histocompatability complex (MHC) II molecules located on the APC surface
- MHC II molecules present this peptides to the T cell receptors
- T cell receptor augmented by additional co stimulatory signals between the APC and T cell
- Co stimulatory signals including ligation of CD 28,CD 80,CD86(B7-2)
- In the presence of appropriate co stimulation and cytokine environment navie CD^{4+} cells (th0) may be induced to differentiate into T helper cells I (Th1)or Th2 clones
- Th1 cells are classically associated with cell mediated functions and the synthesis of complement binding IgG subclasses(Ig G1 and IgG2)

Th 2 cells are important in the synthesis of non complement binding antibodies IgG4 and IgE and cytokines secreted by the effector Th1 (IL-2 and interferon gamma)and Th2 (IL4,5 and 10)clones then direct B cell synthesis of antibodies, which in the case of FVIII, function as inhibitors.[34]

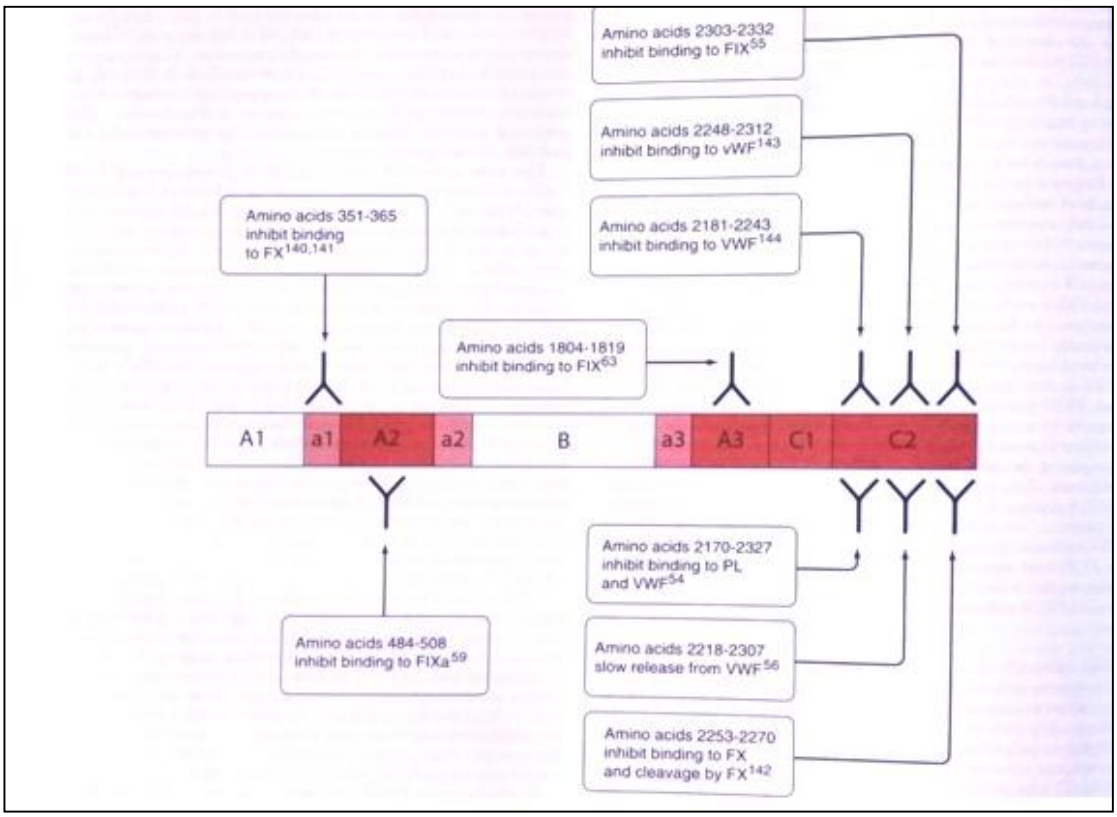


FIGURE.6. FACTOR VIII DOMAINS AND BINDING SITE BY AMINO ACID LOCATION AND EFFECT ON FACTOE VIII

Factor VIII contains three A domains(A1,A2,A3),one B domain and two C domains(C1,C2). Inhibitory antibodies are primarily directed against the A2,A3 and C2 domains which are the interaction sites of FIX, phospholipids and von willebrand factor.[31] and interfering with proteolytic activation of FVIII.[28] these binding sites are essential for Factor VIII to activate the coagulation cascade.[35] Factor VIII procoagulant levels are inhibited by inhibitors in several ways including blocking the binding of FVIIIa to FIXa, FX . Additionally antibodies that interfere with binding to VWF could displace FVIII from V in vivo and increase the clearance of FVIII.[32.] The formation of Factor VIII antibodies in patients with haemophilia A occurs because the

infused Factor VIII is recognised as foreign protein, it will triggering the immune response.[36] Inhibitor is a polyclonal high affinity immunoglobulin G (IgG) that is directed against the FVIII protein.[31] Hemostatic action of Factor VIII is inhibited by these inhibitors.[36] Predominant one is IgG4 which do not fix the complement which has been cited as a reason that immune pathology due to antigen antibody complex is not observed in FVIIII inhibitor patients.[32] Inhibitors developed no tolerance to endogenous FVIII, they may simply react to exogenous FVIII as a foreign or non self protein by forming inhibitor antibodies.[37] The only biological function of FVIII is to become proteolytically activated and participate as a cofactor for FIXa during intrinsic pathway factor activation on phospholipids membranes.[32]

CLASSIFICATION OF INHIBITORS:

Based on the kinetics and extent of inhibition Inhibitors are classified as Type I and Type II.[31] TYPE I inhibitors follow second order kinetics (dose dependant linear inhibition) and completely inactivate FVIII, more common in severe haemophilia. Type II inhibitors have complex kinetics and incompletely inactivate FVIII even after prolonged incubation.[34]Type II inhibitors commonly developed in mild haemophilia or in patients with acquired haemophilia.[31] Another classification based on a patient's peak inhibitor titre after repeated exposure. The international society on thrombosis and haemostasis scientific and standardization committee has recommended that an inhibitor titre of 5BU differentiates low from high responding inhibitors. An antibody titre that is persistently below 5BU despite repeat challenges with FVIII is considered a

low responding inhibitor, and the high titre is when the inhibitor assay has been greater than 5BU at any time. One Bethesda unit is defined as "the amount of inhibitor that will neutralize 50%of 1 unit of factor VIII:C in normal plasma after 2 hrs of incubation at 37°c.[13]

TABLE.1. CLASSIFICATION OF INHIBITOR :

Low response inhibitor	inhibitor titre persistently < 5 BU /ml despite repeated challenges with FVIII concentrates.[82]
High response inhibitors	inhibitor titre ≥5 at any time.[82]
Transient	disappeared spontaneously after at least two consecutive positive detections.[82]

In the absence of FVIII exposure, high responding inhibitors may decrease and may even become undetectable. Classically, when high response titre patients are re exposed to FVIII, their titre will increase over 4 to 7 days. This response is anamnesis and is a hallmark of high responding inhibitor. Low titre inhibitors comprise 25 to 50 % of observed inhibitors and approximately 10% of these are considered transient, disappearing over weeks to months despite continued treatment with FVIII.[36] Haemophilia A patients with a positive ELISA but undetectable inhibitor levels by Bethesda assay have rarely been identified indicating the presence of non inhibitory antibodies.[32]

ACQUIRED HAEMOPHILIA:

Auto immune bleeding disorder involving the coagulation system occur as auto antibodies in non haemophiliacs produce a condition called acquired haemophilia commonly seen in older age group patients of either sex.[32] Incidence of acquired hemophilia is 1to 4 per million/year.[39] Very uncommon condition in children (045per million /year). Incidence of acquired haemophilia A increases with age (14.7 per million /year). Incidence in men and women is similar except in the age range of 20 to40 yrs when the effect of pregnancy results in preponderance in women.[39] 50% patients have underlying conditions ,including auto immune disorders, malignancy, pregnancy. Most haemophilia A inhibitor (allo antibody) recognize both A2 and C2 domains. in contrast most auto antibodies recognize either the A2 or C2 domain, but not both, with the C2 domain is frequently affected. Haemarthrosis is rare, but bleeding is more severe than haemophilia A patients with inhibitor.[39]

PREVALENCE OF FACTOR VIII INHIBITORS:

The prevalence of inhibitors is defined as the "proportion of patients with inhibitors at a specific point in time". The prevalence of inhibitors is thought to be about 5-7%. The incidence of inhibitor development is defined as the number of new cases in a specific period of time. The incidence of inhibitors in individuals with haemophilia A is estimated to be as high as 33%, but only 1-6% in patients with haemophilia B. The reason for the difference

between prevalence and incidence has to do with the disappearance of many transient low-titre inhibitors and successful tolerization of others.[40]

RISK FACTORS FOR INHIBITOR DEVELOPMENT IN HAEMOPHILIA A PATIENTS:

- Genetic mutation :the highest risk is found for null mutations (large deletion, non sense mutation and the intron 22 inversion)
- Family history of inhibitor
- African or Hispanic race ethnicity
- Immunologic factors include the major histocompatability complex class ii system and polymorphism of cytokine (interleukins, TNF α)

TREATMENT RELATED RISK FACTORS :

- Intensity of the first FVIII exposure :surgical procedure, high frequency treatment
- Source of FVIII: plasma derived versus recombinant factor products
- Type of therapy : On demand or prophylaxis

TABLE.2.RISK FACTORS FOR INHIBITOR DEVELOPMENT

RISK FACTORS	EFFECT	REFERENCES
PATIENT RELATED		
MUTATION	Severe haemophilia. Highest risks: null mutation, large deletion, inversion 1 inversion 22	Oldenburg (2006)[60], Gouw et al (2011)[78],
ETHNICITY	2 to 5 fold increased risk associated in patients of Hispanic and African origin compared with Caucasians	Viel et al (2009)[92] Astermark et al (2001)[66]
FAMILY HISTORY	Increased with first degree family history , Incidence with family history 48%,Incidence without family history 15%.	Astermark et al(2001)[66]
AGE	Risk is highest below the age of 5years and increases after the age of 60 yrs.	Gouw et al (2007)[78] Chalmers et al(2007)[42] Santagostino et al(2005)[82] Hay et al(2011)[56]

TREATMENT RELATED EXPOSURE DAYS:	Risk highest during early exposure with a median time of inhibitor presentation at about 10-15 EDs. Risk lower after 150 EDs	Lusher et al(1993)[65] Shapiro et al (2005)[20] Hay et al (2011)[56]
INTENSE EXPOSURE	Risk increased with 5 or more EDs at first treatment	Gouw et al (2007)[78] Chalmers et al (2007)[42]
PROPHYLAXIS	Early prophylaxis is associated with a decreased risk .	Gouw et al (2007)[78]
TYPE OF CONCENTRATE	Severe haemophilia A No evidence of any difference in inhibitor risk between recombinant and plasma derived concentrate	Franchini and lippi (2010)[19] Aledort et al (2011)
SURGERY	Severe haemophilia A Risk increased if surgery combined with an intensive first exposure (>4 ED) compared to first exposure without surgery.	Gouw et al (2009)[78]

GENETIC RISK FOR INHIBITOR DEVELOPMENT:

The important risk factor for development of inhibitor is mutation in FVIII gene.[31] Patients with mutations on their FVIII can generally be divided into two type 1) Severe molecular defects including null mutations, as the FVIII proteins production is completely failed including large deletions(>1exon),[107] non sense mutations, intron 1 and intron 22 inversion. 2)Milder molecular defects including missense ,small deletion, insertion and splice site mutation who have loss of function ,but not complete absence of FVIII protein.[39] Chamer's et al showed the distribution of molecular defects in the structure of FVIII gene were missense mutation (23%), smalldeletion / insertion (13%), splicesitemutation (3%) deletions (2%) inversions (56%), stop mutation (3%).[42] Mutations associated with the overall highest rate of (21-88%) inhibitor formation are null (large deletions, nonsense mutations and intron 22 inversions). Null mutation in factor gene cannot produce Factor VIII protein. Among these the most common severe FVIII mutation is intron 22 inversion. The incidence of inhibitor development in null mutation is 21%. The highest proportion of inhibitor formation (88%) is a seen in large deletion that involves deletion of multiple domains. missense and splice mutations will result in loss of function of FVIII, but retain certain production have a lower risk of inhibitor formation (3-10%).Small deletion or insertions have very low risk of inhibitor formation.[31] Oldenberg et al found that 68.8%of those with large deletions had higher inhibitors compared with only 21.2% with missense

mutation and 30% to with all other mutation types.[36] Tuddenham et al, Antonarkis et al ,says ,major loss of coding information and lack major circulating antigen ,results in 35-40% inhibitors.[43]

INHIBITOR DEVLOPMENT ASSOCIATION WITH RACE / ETHNICITY:

Miller et al says in patients of African or Hispanic descent have an increased risk of inhibitor formation.[31,43] Viel and colleagues demonstrated the difference in the FVIII haplotype between patients of african and recombinant factor VIII . Aster mark et al found African race had two fold increased risk for inhibitor development compared with white population reference group.[34] Chamer's et al studied about the role of ethnic origin in inhibitor development, in his study he showed data on ethnic origin were recorded for 324 out of 348(93%) patients. Two hundred and sixty-two of 324(81%) were of Caucasian origin while 62 of 324(19%) were non-Caucasian. Within the non-Caucasian subgroup 64% were of Asian or Arab origin and only 18% were of African or Hispanic descent. Inhibitor development was analysed for patients in each ethnic group. In another study ,he showed the overall incidence of inhibitor development in Caucasian patients was 53 of 262(20%) when compared with 18 of 62 (29%) in non Caucasians(High titre inhibitors were recorded in 32 of 262 (12%) of Caucasians and 11of 62 (18%) of non-Caucasians. In a recent Japanese study, 26.8% of patients with haemophilia A developed inhibitor.[42.] A large Indian

study of 1285 patients with haemophilia A found that only 6.07% of the patients had inhibitors, although there were remarkable regional variations (the highest prevalence was 20.99%.[44].

FAMILY HISTORY OF INHIBITOR:

The genes for FVIII have high rates of mutation and 30% of patients do not have family history of hemophilia.[45] Charmer's et al studied about the data on family history of inhibitor development, he showed were available for 309 of 348 (89%) patients. Thirty-one of 309 (10%) of patients had a positive family history of inhibitor development in at least one affected male relative. Of those with a positive family history, the overall incidence of inhibitor development was nine of 31 (29%) when compared with 52 of 278 (19%) in those with a negative family history. High titre inhibitors were recorded in six of 31 (19%) of those with a positive family history when compared with 31 of 278 (11%) in those with no family history. These figures demonstrated a trend towards a higher incidence of inhibitor development in those with a positive family history .[42]

INHIBITORS ASSOCIATED WITH SEVERITY:

Commonly the inhibitors been developed in 25-30% of severe haemophilia.[46] Inhibitors most commonly seen in patients with severe haemophilia A with incidence of 30%.[38] among this 60% high titre (>5BU), and the remaining are low titre (<5 BU). Lusher et al says 3-13% occurs in

mild or moderate haemophilia.[31]. The risk of inhibitor development depends on the percentage of circulating Factor VIII activity. The incidence of inhibitor development is 6 fold higher in patients with large deletions.[47] Tuddenham et al 1994 reported that patients with large deletions, stop mutation and inversion showed that inhibitor incidence of 35%. Missense mutation and small deletions have 5 to 8 fold lower incidence in inhibitor development like 4.3% and 7.4% respectively. Mutations which result in a major loss of coding information and lack of circulating FVIII antigen are all associated with similar, high, 35-40%prevalence of inhibitors.[47.] Risk factor for inhibitor formation in mild hemophilia includes later age with first factor VIII exposure. Intensity of the FVIII, family history and the type of genetic mutation may influence the inhibitor development. In mild haemophilia has spontaneous resolution has been reported in up to 60% cases, after a median of 9 months, however 75% of patients with spontaneous resolution experienced anamnesis with repeat FVIII exposure.[31]. The amount mount of exposure to FVIII is the risk of inhibitor development in mild haemophilia.[32.].Severe defects, large deletions in the FVIII gene ,intron 22 inversions, stop mutations are associated with higher risk of inhibitor development than small deletions /insertions, missense mutation or splice site mutations. Patients with severe defects were nearly three times more likely to develop inhibitors compared with patients with low risk mutation.[48] Inhibitor development was more common among patients with severe disease (39/127; 30.7%), compared to patients with mild disease (4/14; 28.6%).[44]

Previously inhibitor development in mild haemophilia is less common than severe haemophilia.[49] With an estimated incidence of 3-13% patients with mild haemophilia form inhibitors against the exogenous infused FVIII and commonly there is inhibitor cross reactivity against the patients endogenous FVIII decreasing a patients baseline FVIII level. This decrease changes the patient phenotype from mild to moderate to severe .Both type I &II inhibitors are present, there appears to be predominance of type II inhibitors.[31]

Sharathkumar et al found inhibitors in milder forms of haemophilia A more commonly arise under conditions in which the immune system is under intense stimulation(suggested that continuous infusion may alter the immunogenicity of the FVIII molecule/or exposure to FVIII is unusually high, for example in the post operative period). Mutations that result in stable abnormal conformation in the FVIII molecules are at particularly high risk for inhibitor formation in mild haemophilia A Thompson et al says Arg593 —> cys mutation is representative of the majority of mild haemophilia inhibitors, in so far as tolerance to both exogenous and endogenous FVIII is lost[36]. Peer linck et al says the inhibitor from patients with the Arg2150->His mutation neutralizes exogenous, but not endogenous ,FVIII.[34]. Risk factors for inhibitor formation in mild haemophilia A are

- ✓ Intensity of the factor VIII exposure
- ✓ Family history of inhibitors
- ✓ Types of genetic mutation

Association between the Arg 593 Cys genetic mutation and an increased risk of inhibitor formation .spontaneous resolution has been reported in up to 60 % of cases after a median Of 9 months 75% of patients with spontaneous resolution experienced anamnesis with repeat FVIII exposure DDAVP should be used preferentially over factor products in those who are responder.[31]

Bi model peak of inhibitor risk in early childhood and old age. Inhibitors develop primarily during early childhood, at the average of 12 yrs. Development may occur as early as 1 to 2 years typically during the first 20 exposure days when inhibitor risk is greatest.[50] The majority of inhibitors developed during childhood period, at an average age of 12 years, the inhibitor development occurs in children with severe haemophilia at an average age of 1-2 years. In severe haemophilia A inhibitors developed at a rate of 6.4 per 1000 years at risk for all ages combined. The rate varied with age, taking values 34.4, 5.2 and 3.8 per 1000 years at risk at ages <5, 5–14 and 15+years, respectively. For patients with moderate/mild haemophilia A the rate of inhibitor development was just over one quarter that for patients with severe haemophilia A of similar age[91]. The mean age at development of inhibitors was 17.7 years (range 6±52 years).[51] The age range of patients with haemophilia A was 1-53 years (median age, 16.0 years).[52] Inhibitor formation is commonly seen in < 5 years age group, rarely seen after 11 years.[53] In children with severe haemophilia A ,the inhibitor development is highest by the age of 5 years, the cumulative risk reaching 16%,the risk reaches 36% by age 75 yrs.[49]

Vast majority (73%) of inhibitors are identified within the first10-20 exposure days to factor concentrates While it is known that most inhibitors develop within the first 10-20 exposure days .[45] Early exposure to FVIII during the first 6 months of life was associated with a higher incidence of inhibitor formation.[54] Whether early exposure to FVIII is related to inhibitor development has potentially important implications for the initial management of infants with haemophilia, particularly those who require treatment at a very young age. The option of delaying FVIII exposure in young infants was explored recently by Rivard et al in a small pilot study in which the aim was to use rFVIIa in place of FVIII until the age of 2 years. Of the 11 infants in this study, six still required FVIII to control bleeding and four subsequently developed inhibitors, which suggests that this is unlikely to be a feasible approach [54,38]. The highest risk of developing inhibitors is observed within the first 50 exposures to FVIII with the risk reducing substantially after 200 treatment days .[43,55]

IMMUNOLOGIC FACTORS:

Astermark et al International Brother Study demonstrated that polymorphisms of the TNF α gene and IL 10 are associated with an increased risk of inhibitor formation.[31] .Explored immunologic factors include the major histocompatability complex (MHC) class II system and polymorphisms of cytokines, TNF α [31] The MHC class II alleles in inhibitor development has a weak association. Astermark et al evaluated the effect of polymorphism in immune response genes on inhibitor development, the association of these

polymorphism with inhibitor formation strongly suggest that, in addition to lack of self tolerance to FVIII.[36.] Polymorphism in the gene coding for IL -10,TNF α, Cytotoxic T lymphocyte antigen 4 have been identified as a genetic factors in the context of Malmo international brother study.[43,36] . Inhibitor development may occur in conjunction with danger signals presented to the immune system.[32] Injury or inflammation at the time of FVIII exposure to send danger signals In the danger model, damaged cells send alarm signals that activate antigen presenting cells, which one amplifying immunologic responses. Although danger model may apply to the overall result of the CANAL study, approximately 20% of subjects still developed an inhibitor in the absence of circumstances that could be associated with these danger signals.[36] Hay et al, Oldenburg et al demonstrate the association between inhibitor risk and HLA haplotypes. The HLA DRB1*1501 / DQBI*0602 / DQAI*0102 haplotype was associated with inhibitors in factor VIII gene intron 22 inversion positive patients and the HLA –DRB1*01 / DQA1*0101 / DRB1*0501 haplotype associated with inhibitors in intron 22 inversion negative patient.[36] Major histocompatability complex molecules which play a central role in the cellular cascade leading to antibody formation, have been evaluated as potential co determinants.[48].Two studies detected a weak influence from MHC i/ii genotypes. No association was found in the MIBS cohort. The MIBS study also showed that there was a strong association between inhibitor development and a polymorphism located in the promoter region of the IL-10 gene.[57]

RISK FACTORS RELATED TO TREATMENT:

AGE AT FIRST EXPOSURE TO FACTOR VIII:

In the CANAL cohort study, the risk of inhibitor development in patients treated with FVIII before the first month of age was 41% compared with 18% in those who started the treatment after the age of 18 months.[48] That first replacement therapy at an early age may increase the risk of inhibitor formation. Lorenzo et al reported that cumulative incidence of inhibitors at 3 years was significantly higher in those initiating therapy before 6 months of age compared with patients starting with treatment between 6 and 12 months of age or those treated at age > 12 months (41%vs 29%and 12% respectively) van der bom et al who reported that the earlier the exposure to FVIII in infancy (at the age of 6 months) the higher the risk of developing inhibitors later in life.[43]. Recombinant products have an increased risk of inhibitor formation over that of plasma derived products. The increased immunogenicity to be secondary to alterations in post translational modifications of FVIII and a lack of von wile brand binding. in previously untreated patients the inhibitor formation is more common in recombinant (28.7%) than plasma derived(10.3%).[31].Rivard et al reported that the use of recombinant activated FVIIa on demand in patients with severe haemophilia A decrease the risk of developing FVIII inhibitors by postponing the first exposure to FVIII concentrates until after 2 years of age. [43]

INTENSITY OF FACTOR VIII AT FIRST EXPOSURE:

The intensity of the first FVIII exposure leads to immunologic danger signals that stimulate antigen presenting cells and amplify an immunologic response which could promote inhibitor development.[31] The inhibitor development can be influenced by the circumstances in which FVIII is used, in Previously untreated, patients(PUP),65% of those in which surgery was the first indication for FVIII developed an inhibitor compared with approximately 23% in those with other indications for first treatment those who received 5 or more consecutive days of FVIII at the time of their first exposure,56% developed inhibitor, compared with 19%in the group that received fewer than 3 consecutive days of FVII.[36] Gouw et al intensive treatment periods (peak treatment moments, and surgical procedures) were shown to increase the risk of inhibitor formation. Reduced duration between exposure days was significantly associated with increased risk of inhibitor development.[43] The highest risk of inhibitor development during the first 50 days of exposure .[58]

Sharathkumar et al found that the incidence of inhibitor development was more than four times higher in patients administered with full intense FVIII therapy (administered via continuous infusion) compared with patients receiving bolus injection (57% vs 14% respectively).[45] Sharathkumar et al in their retrospective study they found out patients with mild haemophilia who had received 6 or more consecutive days of FVIII ,inhibitors developed more

frequently in patients receiving continuous infusion compared with bolus injections(57% vs 0%).[36]

Less frequent exposure to FVIII concentrate in patients with mild haemophilia accounted for a lower incidence of inhibitor development CANAL study ,dose of 35 -50 IU/kg over five consecutive days was associated with 1.4 times the risk than normal dose ,35 IU/kg. This increased to 3.3 times the risk of a normal dose when FVIII was administered at doses of 50IU /Kg.[59] Association between inhibitor development and number of exposure days to FVIII was also examined in the CANAL Study .Higher number of consecutive exposure days increased the inhibitor development in severe haemophilia patients .In a multicentre cohort study shorter duration between exposure days increased the risk of inhibitor development. biological evidence indicates that higher dose of FVIII will lead to an increased risk of inhibitor development .Major injuries and surgeries cause tissue damage and inflammation .Damaged cells from injured areas send danger signals which activate FVIII antigen presenting cells, up regulating. Co stimulatory signals to T lymphocytes. Both FVIII expressing antigen presenting cells and T lymphocytes enhance the formation of antibodies to FVIII in B lymphocytes.

FACTOR VIII CONCENTRATES:

There are 2 types of Factor VIII concentrates(plasma-derived factors and recombinant factors), which are associated with varying rates of inhibitor

formation.[42].Availability of different types of these products, the influence of the type of Factor VIII concentrate in PUPs with severe haemophilia A is controversial.[60,61,62].The role of pd FVIII in the development of inhibitor with a cumulative incidence of inhibitors ranging from 20.3% to 33.0% in PUPs exposed to different brands of low or intermediate purity pd FVIII. [88] Rate of inhibitor formation in previously untreated patients with haemophilia A were similar to that observed with full length rFVIII concentrtes.[24] In 2006 , Gringeri and colleagues retrospectively evaluated the occurrence of inhibitors in PUPs with severe haemophilia A treated with plasma derived VWF containing FVIII concentrates and found an inhibitor incidence of 9.8%.Several other studies of patients treated with a single plasma derived high purity anti haemophilic factor concentrate containing vWF showed the incidence of inhibitors in the range from 0% to 12.4%.[61,62,63,64] Most of the current high purity pd FVIII products carry almost 0% risk of inhibitor formation.[64.65] There are data supporting the protective effect of vWF , a carrier protein of FVIII which is present in a large amount in most pd FVIII products , but not in rFVIII. While study by schwarzinger et al 1987; Rasi &ikala,1990; sultan et al 1992;gave observations that high purity Factor VIII concentrates caused more inhibitors than traditional intermediate purity products.[74.] Inhibitor development was most common in the recombinant subgroup (14/43; 32.6%), which was followed by the plasma-derived subgroup (19/59; 32.2%), the group with multiple products (6/22;27.3%), and the fresh frozen plasma group (4/18; 22.2%). However, these differences were not statistically significant (P=0.883).Inhibitor

development is lower than patients treated with low purity and intermediate purity.[32] Plasma-derived FVIII concentrates might be associated with a lower incidence inhibitor development due to the protective effect of their von willebrand factor which would mask the epitope sites of inhibitors on the FVIII molecule or would prevent FVIII endocytosis by dendritic cells.[58] Gouw et al in his study ,he reported that no difference between low von willebrand factor content and high von willebrand factor content.[25] Recombinant products are available in different generation which are First-generation recombinant FVIII products . The recombinant FVIII concentrates that use animal-derived proteins in the cell culture medium and have human serum albumin added to stabilize the final formulation. Second-generation recombinant FVIII products: The recombinant FVIII concentrates that use animal-derived proteins in the cell culture medium but have no human serum albumin added to the final formulation .[58] The incidence of inhibitor formation in PUPs with severe hemophilia A ranges from 2.7% to was51.8% with plasma-derived FVIII and from 7.7% to 41.9% with recombinant FVIII concentrates .No statistically significant differences in cumulative inhibitor rate were found between plasma-derived FVIII and any recombinant FVIII preparations.[58] Rates were higher in patients treated with first-generation recombinant FVIII (0.31; 95% CI, 0.25–0.37) than among those treated with second-generation recombinant FVIII products (0.18; 95% CI, 0.09–0.31). We also found a higher prevalence of inhibitors among patients who were receiving recombinant factors, and this result agrees with the findings from our previous studies.[42]

Meta-analysis conducted by Iorio and colleagues [87]. These authors identified 2094 patients, from 24 retrospective and prospective studies, among whom 420 developed inhibitors. The pooled incidence inhibitor rate was 14.3% for plasma-derived FVIII concentrates and 27.4% for recombinant FVIII products ($p < 0.001$), although the difference lost statistical significance at multivariate analysis. Franchini et al was observed similar results when the analysis was restricted to the 19 prospective studies (9.1% for plasma derived FVIII concentrates and 23.7% for recombinant FVIII products, $p < 0.001$).[66] By contrast, their meta-analysis showed a non-statistically significant difference (weighted means, 21% with plasma-derived FVIII versus 27% with recombinant FVIII products) in inhibitor incidence. Recombinant products have an increased risk of inhibitor formation over that of plasma derived products.[31]The increased immunogenicity to be secondary to alterations in post translational modifications of FVIII and a lack of von wile brand binding. In previously untreated patients the inhibitor formation is more common in recombinant (28.7%) than plasma derived(10.3%).[31] Also in the meta-analysis by Iorio and colleagues, the statistical significance in inhibitor incidence between prospective studies involving plasma-derived or recombinant FVIII concentrates disappeared when only high-titre inhibitors were considered (6.0% with plasma-derived FVIII versus 19.4% with recombinant FVIII products, $p = 0.195$). This meta analysis showed the lowest inhibitor incidence rate (11%) was found with the second generation recombinant FVIII concentrate.

MODE OF TREATMENT : PROPHYLAXIS AND ON DEMAND

'The definition of prophylaxis is the regular infusion of factor VIII concentrate with the aim of preventing bleeding, starting within first two years of life'.[67] Several different prophylaxis regimen, which are differentiated by dose and frequency of factor administration. Malmo regimen full dose prophylactic regimen involves administration of 25 to 40 U/kg of FVIII every other day (minimum 3 days /week).the "intermediate –dose" prophylactic dose regimens involves the administration of 15 t0 25 U/kg two or three times a week. Low dose prophylactic regimen involves 10 to 15 U/ kg given one or two times a week.

TABLE.3.TREATMENT REGIMEN

Full dose regimen	25 to 50U/kg ,3days(every other day) / week
Intermediate dose regimen	15 to 25 U/kg two to three times /week
Low dose prophylaxis	10 to 15 U/kg one or two times/week

Prophylaxis regimens reduce the risk of inhibitor development compared with bolus on demand treatment in terms of exposure to FVIII .Owing to the similarities in terms of genetic mutations and age in the prophylaxis and on demand groups , the author concluded that on demand therapy represents a clear risk factor for the development d of inhibitors. An

univariate analysis was used to demonstrate that commencing prophylaxis before the age 35 months carried an inhibitor risk of 28% compared with 56% risk in patients with on demand therapy. Danger theory of tolerance proposes that the immune system responds to danger signals from both exogenous and endogenous sources. If an antigen is not itself perceived as dangerous and no other danger signals- such as cell necrosis or tissue injury are present ,tolerance normally occurs rather than an immune response. prophylactic regimen may offer a protective regimen offer a protective effect since the patient is treated in the absence of any additional danger signals, whereas on demand administration of FVIII may be perceived as dangerous doe to danger signals from ongoing bleeding episodes or during physiological stress such as surgery.[48] The prophylactic treatment was initiated in Sweden to prevent the bleeding episodes and minimizing the impact of arthropathy.[10] According to WHO&WFH starting the prophylactic treatment for the child with severe haemophilia At an early age is the optimal therapy." Scientific and standardization committee (SSC) of the International Society on Thrombosis and haemostasis (ISTH) defines the primary prophylaxis is a continuous therapy starting after the first joint bleed and before the age of 3 years". Secondary prophylaxis can either be continuous long term treatment started after two or more joint bleeds or after the age of 3 years. Manco- Johnson et al showed the median haemorrhages of children undergoing prophylactic treatment was 1.2, compared with 17.1 in on demand group. Aster mark et al suggested if the prophylaxis was started before two years of age, significantly

reduce the joint damage. The risk of ICH is 20-50 times higher in a person with hemophilia who is on demand therapy. Starting the treatment as prophylaxis or the first 20 exposure days have a decreased risk to develop inhibitors compared to on demand therapy. Even though the patient receiving the prophylaxis during the first 20 exposure days ,the inhibitors will develop ,because of the type of mutation, family history of inhibitors and other genetic risk factors. SPINART study compared the on demand therapy and prophylactic based on the total bleeding episodes per year(27.9 versus 0), the median number of total bleeding episodes(54.5 versus 0), study showed the bleeding episodes were significantly lower with prophylaxis than with on demand treatment . Primary prophylaxis was successfully pioneered in Sweden and then adopted in other countries, achieving the goal of preventing the majority of bleeding episodes and further reducing the impact of arthropathy.[68]

DIAGNOSIS AND INVESTIGATIONS OF FACTOR VIII INHIBITORS:

Inhibitor is suspected when a patient has a poor clinical response to concentrate or lower FVIII levels than expected after concentrate infusion. it is crucial to detect the inhibitor as early as possible is to minimize anamnesis response , limit the unnecessary exposure to sub optimal treatment, if the inhibitor does not rise above 10 BU /ml, allow immune toleration induction to be started without delay .

INHIBITOR TESTING IS REQUIRED:

- before elective invasive procedures when the clinical response to concentrate is sub optimal
- before and after switch of concentrate
- 2-3 weeks after intensive treatment (≥5EDs)
- If any surgery is going to be performed for mild or moderately affected patients

INHIBITOR SCREENING FOR SEVERE HAEMOPHILIA A:

- At least every third ED or every 3 months if concentrate exposure has occurred (whichever is sooner) until 20 EDs have been achieved
- After that every 3-6 months until 150 EDs
- Inhibitors may occur at any age and incidence increases again after the age of 60 years therefore testing should continue 1-2 times a year indefinitely

INHIBITOR SCREENING FOR MILD/ MODERATE HAEMOPHILIA A

- Should be tested annually if exposed to concentrate
- After any intensive exposure (≥5 EDs)
- Surgery

TREATMENT FOR HAEMOPHILIA A PATIENTS WITH INHIBITOR

Aim of the treatment is to achieve a therapeutic level of FVIII to maintain the haemostasis. Choice of treatment product based on titre of inhibitor ,clinical response to product.

FOR LOW TITRE INHIBITOR:

Patients with a low titre inhibitor may be treated with factor replacement at a much higher dose, to neutralize the inhibitor with excess factor activity and stop bleeding.[9]

FOR HIGH TITRE INHIBITOR:

Kurczynski and penner used an Factor VIII bypassing agents (FEIBA), prothrombin complex concentrate (PCC) to circumvent the hemostatic requirement for FVIII . 1975 ,the PCCs contain more concentration of FVII ,showed more effectiveness than the products contains lower concentration of FVII .Since activated factors in the PCCs were thought to be responsible for the hemostatic effect. Then activated PCCs were prepared by "controlled activation" of the original unactivated prothrombin complex concentrate. During preparation the Factor VII is activated to factor VIIa. Factor VIIa in the activated PCCs was considered to be the major bypassing agent. Difficulty in preparing the plasma derived Factor VIIa, resulted in the development of recombinant factor VIIa (rFVIIa).The half life of rFVIIa to be 2.60 to 2.84

hours.[70] Lisman and De Groot et al describes the Mechanism of action of FVIIa as bypassing agent .They said tissue factor pathway is required for rFVIIa will bind to activated platelets and directly activate FX to FX, this mechanism not only accelerates the clot formation also inhibits fibrinolysis by activation of thrombin activatable fibrinolytic inhibitor (TAFI) .The recommended dose is 90µg/kg given intravenously every 2 hrs until bleeding stops.[70] Prophylaxis with daily doses of rFVIIa shown to decrease spontaneous joint bleeds. Monitoring of rFVIIa with either thromboelastography or the thrombin generation is necessary.

IMMUNE TOLERANCE INDUCTION:

The induction of immune tolerance (IIT), pioneered by H.H Brackman.[27] Before immune tolerance induction therapy ,high responding patients should avoid FVIII products, to allow inhibitor titres to fall and to avoid persistent anamnestic response.[9] Repeated doses of FVIII concentrate, along with infusions of prothrombin complex concentrate, were given until the inhibitor disappeared and the half life of FVII was normalized .5-10% of the inhibitors persist even after the immune tolerance induction, render the patients resist to Factor viii replacement.[56] International Immune Tolerance Registry (IITR) describes the factor influencing the outcome of immune tolerance induction are ,the daily factor VIII dose ≥ 200IU/Kg/day was associated with more favourable outcome, particularly in patients with inhibitor titre >10BU/ml . The success rate with current regimens is in the 70±10%.

MATERIALS AND METHODS

This is a cross sectional study done on patients who were diagnosed as Haemophilia A attending Department of Medicine, Royapettah Government General Hospital and the laboratory work up was done at Department of Transfusion Medicine The TN Dr M.G.R Medical University.

Aim of our study is to find out the prevalence of Factor VIII inhibitors in haemophilia A patients those who are receiving plasma derived Factor VIII therapy. The study was done over a period of one year from July 2016 to June 2017.During this period we studied a total of 90 patients with haemophilia A . Factor VIII level estimation, inhibitor screening assay and Bethesda assay was done at Department of Transfusion Medicine The TN Dr M.G.R Medical University.

STUDY POPULATION:

Patients with Haemophilia A who were diagnosed on the basis of clinical features and Factor VIII assay and received plasma derived Factor VIII and blood components (FFP and cryoprecipitate) were included in our study. The study protocol was approved by the ethical committee of The TN Dr M.G.R Medical University and Ethical committee of Kilpauk Medical College. Sample size was calculated by using the formula $Z\alpha\ (1/2)^2\ Pq/d^{2.}$. According to the formula the sample size was 90.

INCLUSION CRITERIA:

All patients those who were diagnosed as haemophilia A and receiving plasma derived Factor VIII therapy in the Hemophilia treatment centre at Royapettah Government general hospital.

EXCLUSION CRITERIA:

Patients those who are diagnosed as hemophilia B, already inhibitor developed patients those who are on rFVII therapy and the patients who are not willing to participate in the study are excluded.

METHODOLOGY:

Complete details regarding the patient which includes name, age , sex, gender, IP number, clinical diagnosis of the patient and the history related to family history of haemophilia A, family history of inhibitor and the treatment history including age at which exposed to Factor VIII, number of exposures to Factor VIII, interval between the exposure days, dose of Factor VIII given were obtained by questionnaire given to the patient. The laboratory work up including the quantitative assessment of Factor VIII, mixing study and the inhibitor screening was done for the patients those prolonged aPTT was not corrected by mixing study, then quantitative assessment of inhibitors (Bethesda study) by using the coagulation analyser named Hemostar 2 channel (from Tulip diagnostics) were done at Department of Transfusion Medicine, The TN Dr M.G.R Medical university .

SAMPLE COLLECTION:

3 ml venous blood sample was collected in citrate tubes (3.2% tri sodium citrate). The blood was mixed with sodium citrate anticoagulant in proportion of blood to citrate as with 9:1.sample was collected within one minute of tourniquet application without much venous stasis and sample was processed immediately. Platelet poor plasma (PPP) was prepared by double centrifugation of a sample at 1700 g for 10 mts at room temperature. Test was done within four hours of collection. If any delay to perform the test , the platelet poor plasma can be frozen at -30°C for 2 months or up to 6 months if stored at -70°C[9].For transportation the sample should be shipped in dry ice (-70°c) to maintain the sample frozen for the required transport time.[19]

PROCEDURE:

APTT

- Pre warm APTT reagent(Liquecelene E, Tulip diagnostics) ,$CaCl_2$ at 37°c for at least 10 minutes
- Pipette 100µl of test plasma into test cuvette
- Incubate exactly for 1 minute
- Add 100 µl APTT reagent and incubate exactly for 3 minutes
- Add 100 µl $CaCl_2$
- Record the clotting time in seconds
- Normal range :28-36 seconds

FACTOR VIII ASSAY

One-stage FVIII: C Assay

- Pipette 100µl of test plasma and 900 µl of Owren's Veronal Buffer into test cuvette
- Take 100µl of diluted test plasma, add 100 µl Factor VIII deficient plasma(Factor VIII deficient plasma ,1 ml, Tulip diagnostics) and start test
- Incubate exactly for 1 min
- Add 100 µl APTT reagent
- Incubate exactly for 3 min
- Add 100 µl $CaCl_2$ record the value in percentage.
- Prepare and run the standards
- Plot clotting time obtained with each standard dilution against % activity using log – log graph
- Factor VIII concentration is expressed in iµ /l or % activity

MIXING STUDY:

- Take 100µl of test plasma, add equal volume (100µl) of pooled normal plasma
- Keep the mixture for incubation at 37°c water bath for 2 hrs
- Run the aPTT test for this incubated mixture of test plasma and pooled normal plasma
- If the aPTT is prolonged, and normal plasma fails to correct the APTT ,an inhibitor should be suspected.[4,13]

INHIBITOR SCREENING:

REQUIRMENTS:

- Pooled Normal plasma
- Test Plasma
- Reagents for APTT ,Calcium Chloride

Method

- 3 Plastic Tubes are prepared – A,B and C
- Put 0.5 ml normal plasma into tube A,
- 0.5 ml test plasma into tube B and
- 0.5 ml each of normal and test plasma into tube C
- Incubate for 60 minutes at 37^0C in water bath
- Make a 50:50 mix from tubes A and B – this is tube D, fresh mix
- Perform an APTT in duplicate in tube no C, incubated mix
- Perform an APTT in duplicate in tube no D, fresh mix
- If negative result, repeat the test at the end of 2 hour
- Results/Interpretation
- If Difference between fresh mix and incubated mix is more than 5 sec indicates the presence of inhibitors. If inhibitor screen positive proceed to inhibitor assay.[10]

FACTOR VIII INHIBITOR ASSAY (BETHESDA ASSAY)

Principle:

A Bethesda unit is defined as the amount of inhibitor which will neutralise 50% of one unit of added factor VIII: C in normal plasma after 2 hours of incubation at 37^0C.[13]

Requirements :

- Owren's Buffered Saline with pH (7.4)
- Pooled normal Plasma
- Calcium Chloride
- Factor VIII deficient plasma
- APTT reagent
- Prepare doubling dilutions ($^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$, ... up to $^1/_{1024}$) of test plasma in plastic tubes in 150 µl volumes using Owren's buffer as diluents.
- Label 12 glass tubes
- Tube 1 - 150µl control pnp + 150µl of buffer
- Tube 2 - 150µl test plasma + 150µl of control plasma
- Tube 3 – 12- 150µl of respective diluted test plasma from ($^1/_2$ - $^1/_{1024}$) + 150µl of control plasma in all tubes.
- Cap, mix by inversion and incubate all tubes at 37^0C for 2 hours

- Perform a Factor VIII assay on all incubation mixtures by the usual factor VIII assay method but use the tube setup as standard as 100 % activity.

RESULTS / INTERPRETATION:

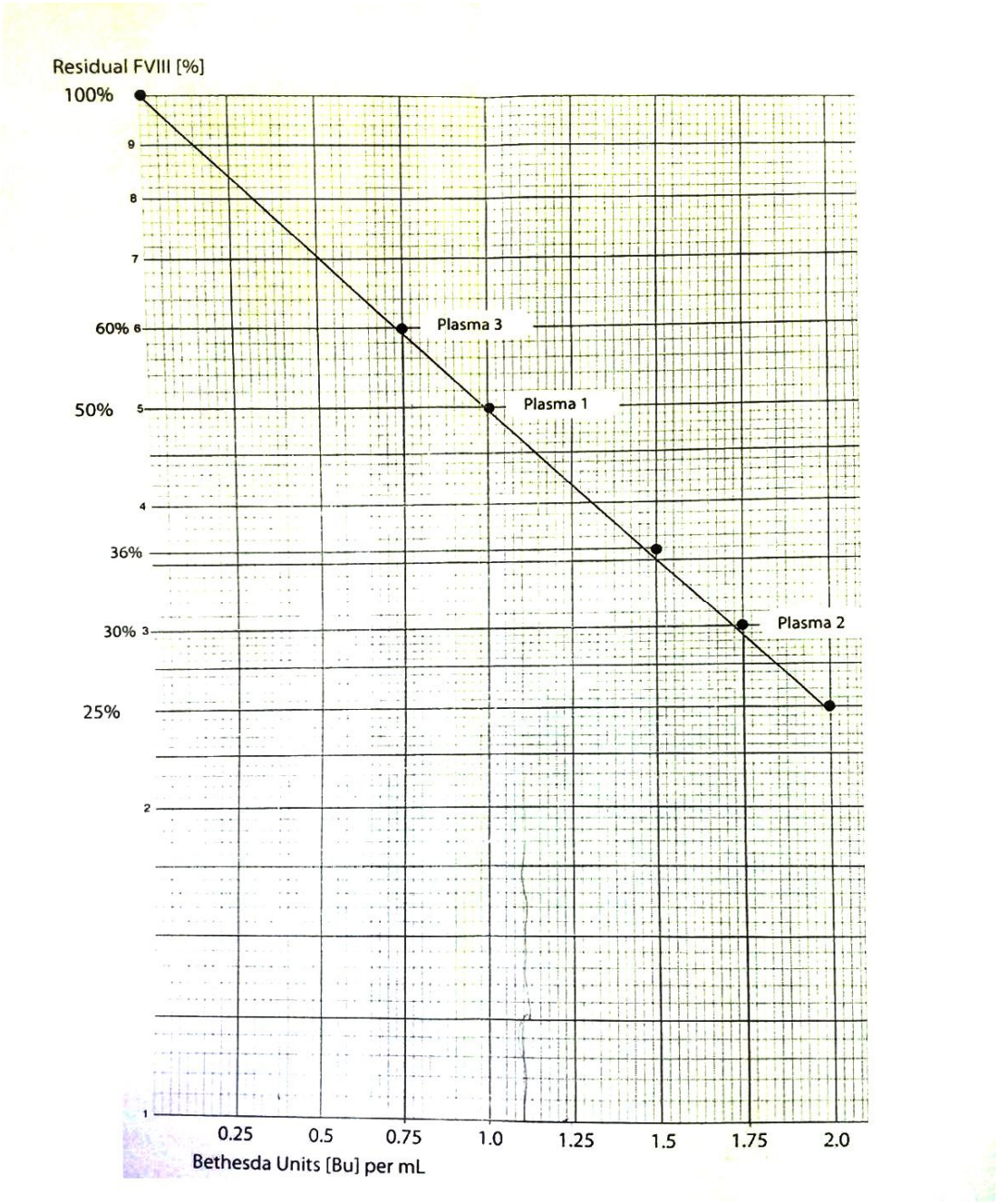
Calculation of Inhibitor Level :

- Factor VIII activity of the control and the patient incubation mixtures are determined from Factor VIII assay curve.

- Residual Factor VIII activity is determined using the Factor VIII activity of the control and dilution of patient plasma having a Factor VIII activity that yields a residual Factor VIII activity greater than 25% lesser than 50%.

$$\text{Residual Factor VIII activity} = \frac{\text{Factor VIII activity(patient)}}{\text{Factor VIII activity (control)}} \times 100$$

- Residual Factor VIII activity is converted to BETHESDA UNIT Factor by using a standard chart.



The Y axis is a log scale and the X axis is a linear. Residual FVIII is plotted on the Y log axis and BU titre on the linear X axis .Derive the inhibitor titre from the graph and multiply by the dilution to give the final titre.

RESULTS:

Table .4.DISTRIBUTION OF PATIENTS ACCORDING TO SEVERITY

CLASSIFICATION	FREQUENCY	PERCENT (%)
Mild	4	4.4
Moderate	27	30.0
Severe	59	65.6
Total	**90**	**100.0**

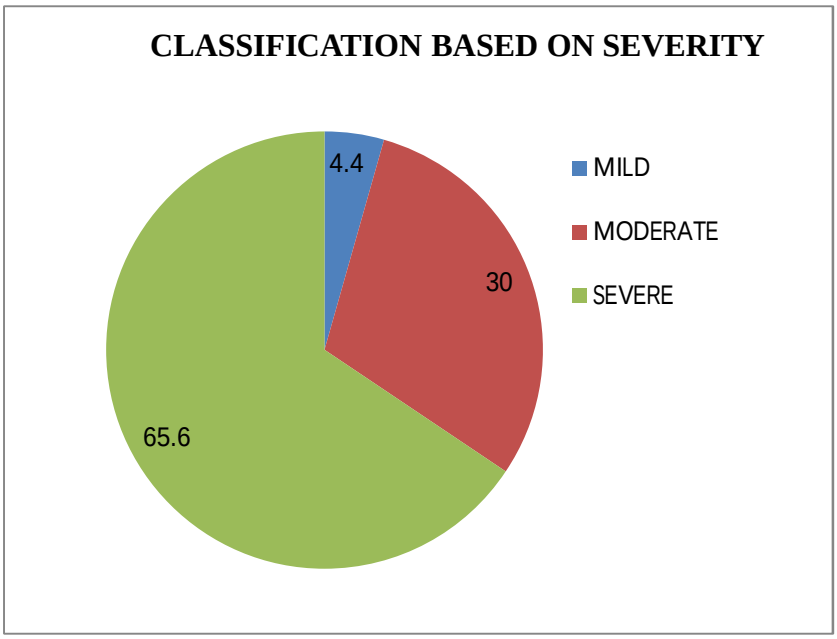


Figure.7.Classification based on Severity

Patients with haemophilia A were classified into 3 groups based on the FVIII levels. In our study out of 90 patients , 59(65.6%)were in the severe group, followed by 27(30%),4(4.4%) patients in moderate group and in the mild group respectively.

Table.5. DISTRIBUTION OF AGE OF DIAGNOSIS ACCORDING TO SEVERITY

Age of diagnosis	Mild	Moderate	Severe	p value
<1year	0	4	31	0.000
1-6years	1	14	25	
7-14years	1	4	2	
>14 years	2	5	1	

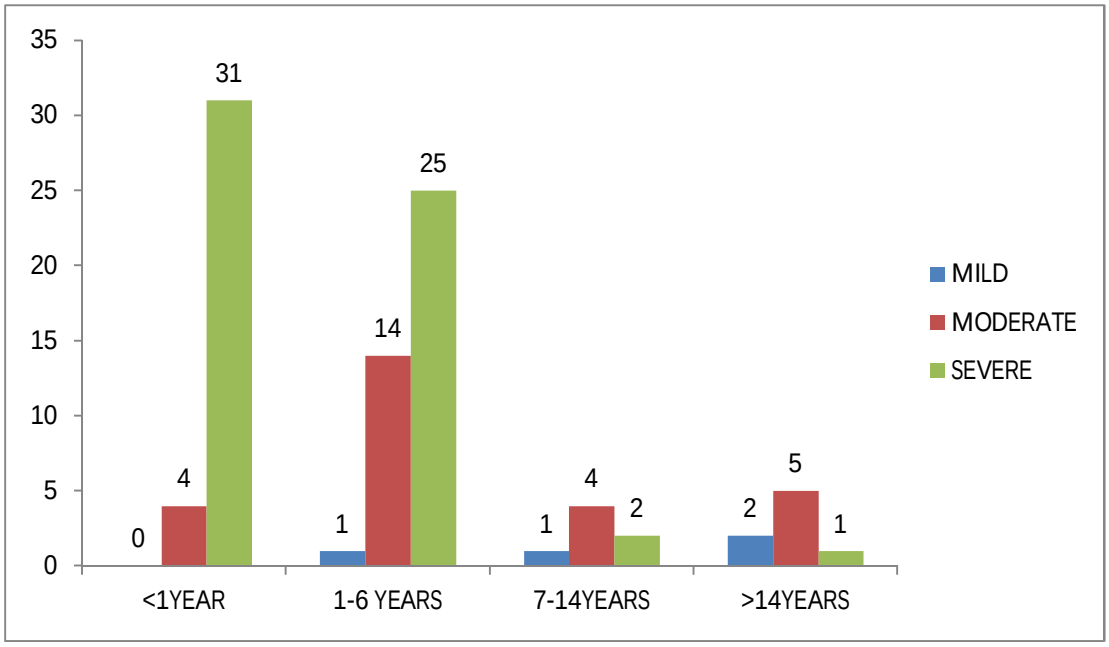


FIGURE.8.AGE OF DIAGNOSIS ACCORDING TO SEVERITY

Out of 90 patients screened, 59 were Severe haemophilia . Out of 59, 31 were diagnosed before 1year of age .Out of 27 moderate haemophilia A patients 4 were diagnosed before 1year of age group. All (4) mild hemophilia patients were diagnosed after 1 year of age group. This association is statistically significant with **p value of 0.000**.

Table.6 .ASSOCIATION BETWEEN FAMILY HISTORY OF HAEMOPHILIA A & SEVERITY OF HEMOPHILIA A

Family history	Mild		Moderate		Severe		Pvalue
	Frequency	%	Frequency	%	Frequency	%	
YES	2	3.84%	9	17.30%	41	78.84%	0.004
NO	2	5.26%%	18	47.36%	18	47.36%	

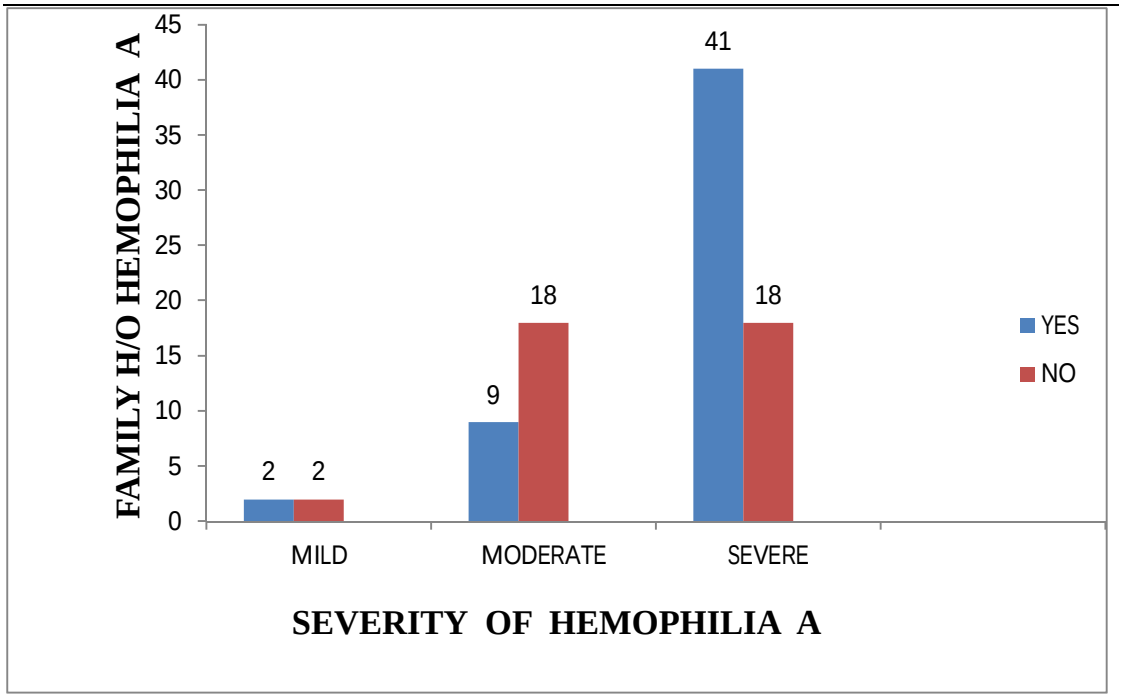


FIGURE.9.FAMILY HISTORY ACCORDING TO SEVERITY

Family history of haemophilia A was highest among in severe haemophilia A patients. This association was statistically significant with **p value of 0.004.**

CLINICAL MANIFESTATIONS OF HAEMOPHILIA A PATIENTS & ASSOCIATION WITH SEVERITY:

Table.7. SPONTANEOUS BLEEDING & SEVERITY OF HAEMOPHILIA A

Spontaneous bleeding	Mild		Moderate		Severe		p.value
	Frequency	%	Frequency	%	Frequency	%	
YES	0	0%	17	22.4%	59	100%	0.000
NO	4	100%	10	71.4%	0	0%	

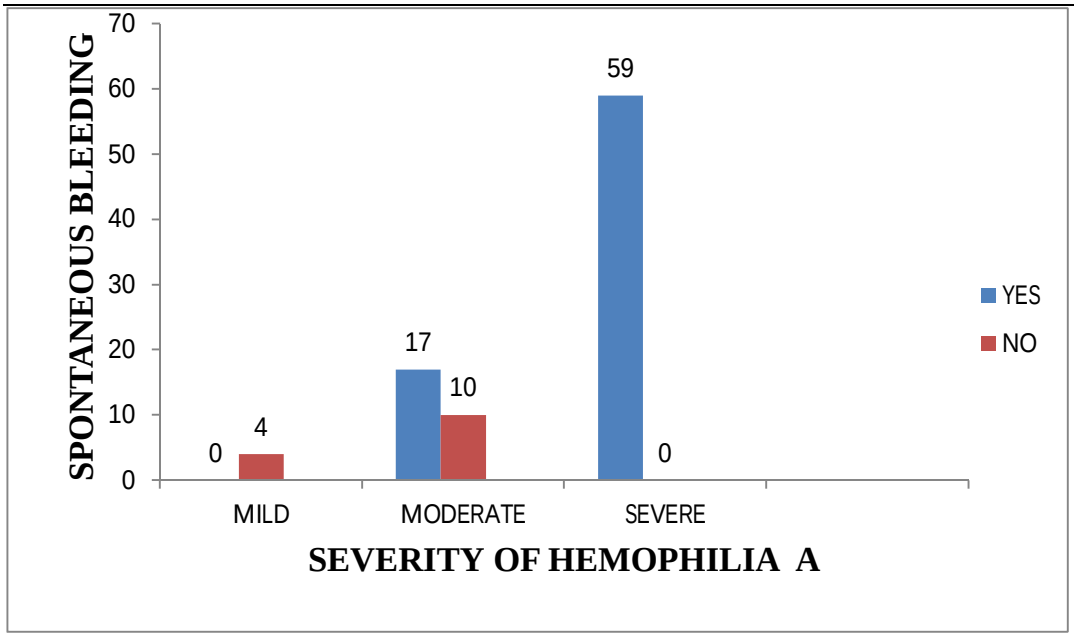


FIGURE.10.SPONTANEOUS BLEEDING & SEVERITY OF HAEMOPHILLIA A

All severe haemophilia A Patients had the history of spontaneous bleeding. This is statistically significant with **p value of 0.000.**

Table.8.GUM BLEEDING & SEVERITY OF HAEMOPHILIA A

Gum bleeding	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	3	3.7%	24	29.3%	55	67.1%	. 223
NO	1	12.5%	3	37.5%	4	50.0%	

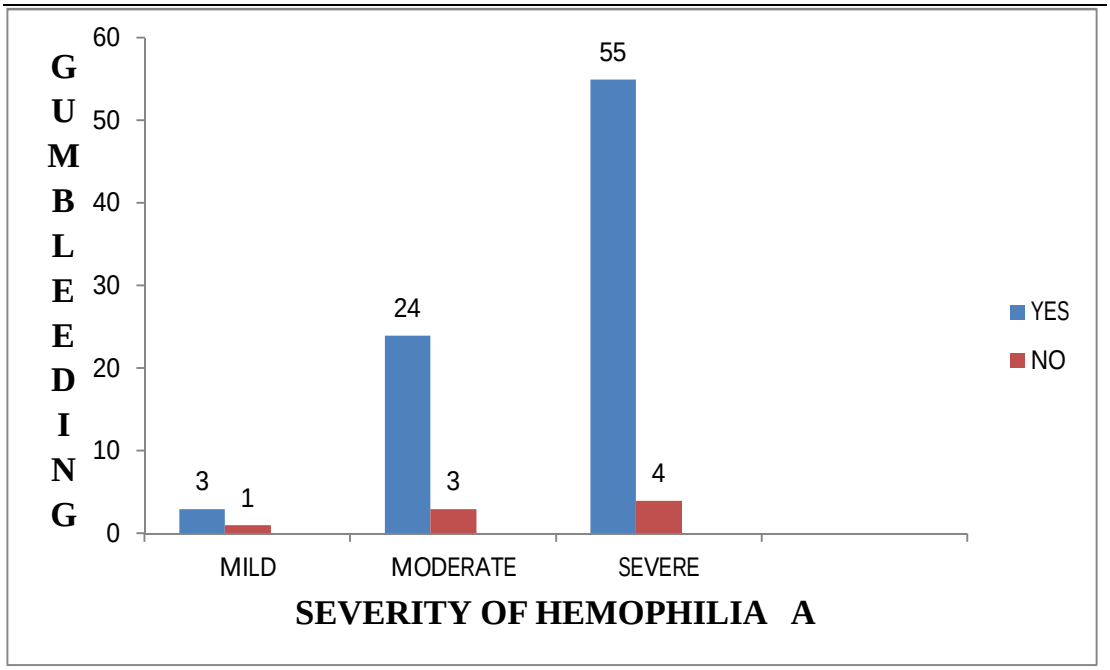


FIGURE.11.GUM BLEEDING & SEVERITY OF HAEMOPHILIA A

Out of 59 severe haemophilia A patients , 55(67.1%) had history of gum bleeding ,followed by moderate (29.3%),and mild (3.7%) haemophilia A.

Table.9.ASSOCIATION BETWEEN PATIENTS WITH EPISTAXIS & SEVERITY OF HEMOPHILIA A

Epistaxis	Mild		Moderate		Severe		p value 0.025
	Frequency	%	Frequency	%	Frequency	%	
Yes	1	2.3%	9	20.5%	34	77.3%	
No	3	6.5%	18	39.1%	25	54.3%	

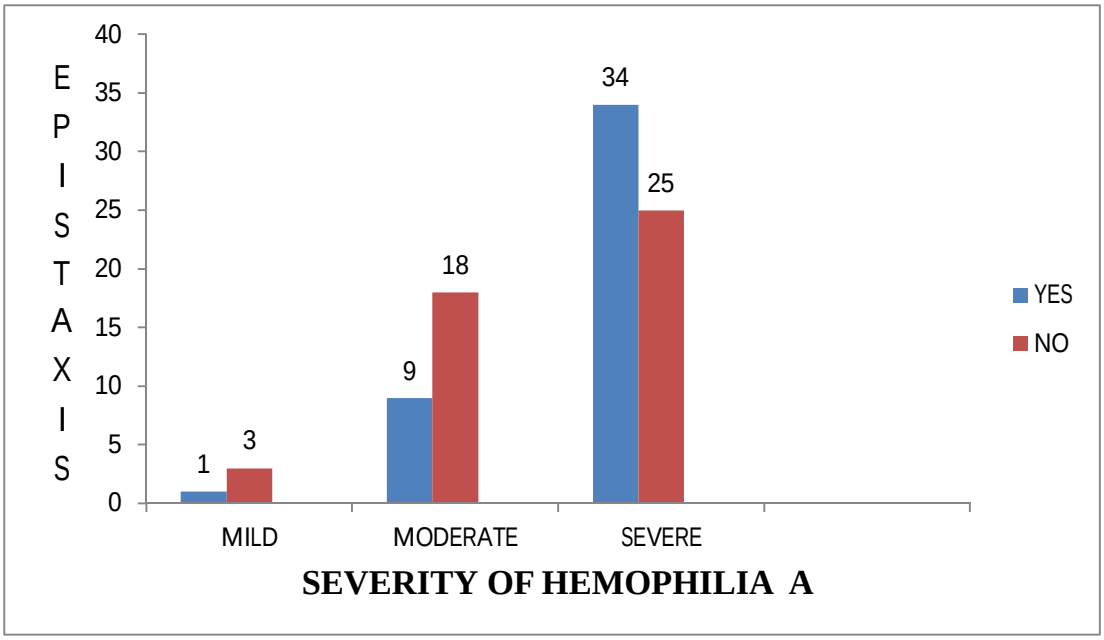


FIGURE.12.EPISTAXIS &SEVERITY OF HAEMOPHILIA A

Out of 90 patients were screened , 44 had the history of Epistaxis. Among the 44, 34 (77.3%) belonged to severe haemophilia A, followed by moderate (20.5%) and mild haemophilia A (2.3%).

This association is statistically significant with **p value of 0.02.**

Table.10. ASSOCIATION BETWEEN PATIENTS WITH HEMARTHROSIS & SEVERITY OF HEMOPHILIA A

Hemarthrosis	Mild		Moderate		Severe		P value
	Frequency	%	Frequency	%	Frequency	%	
YES	2	2.3%	26	30.2%	58	67.44%	0.008
NO	2	50%	1	25%	1	25%	

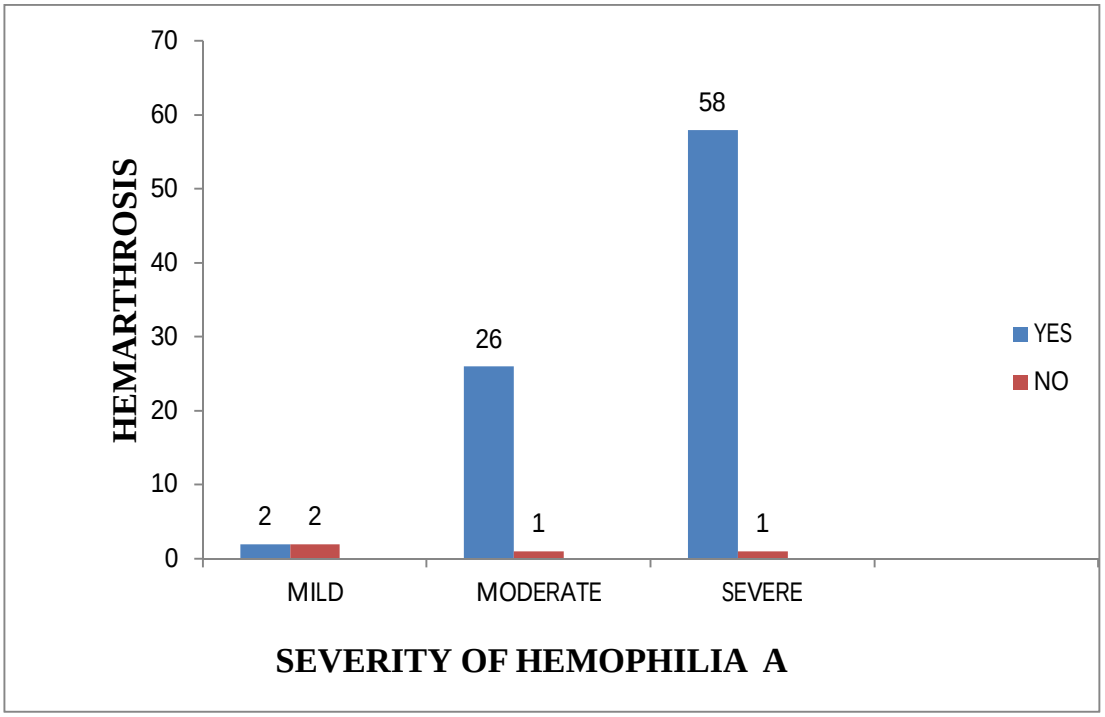


FIGURE.13.HEMARTHROSIS& SEVERITY OF HAEMOPHILIA A

In our study group, hemarthrosis was highest among severe group. This association is statistically significant with **p value of 0.008.**

Table.11. ASSOCIATION BETWEEN PATIENTS WITH HEMATOMAS & SEVERITY OF HAEMOPHILIA A

Hematomas	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	2	2.7%	21	28.8%	50	68.5%	0.112
NO	2	11.8%	6	35.3%	9	52.9%	

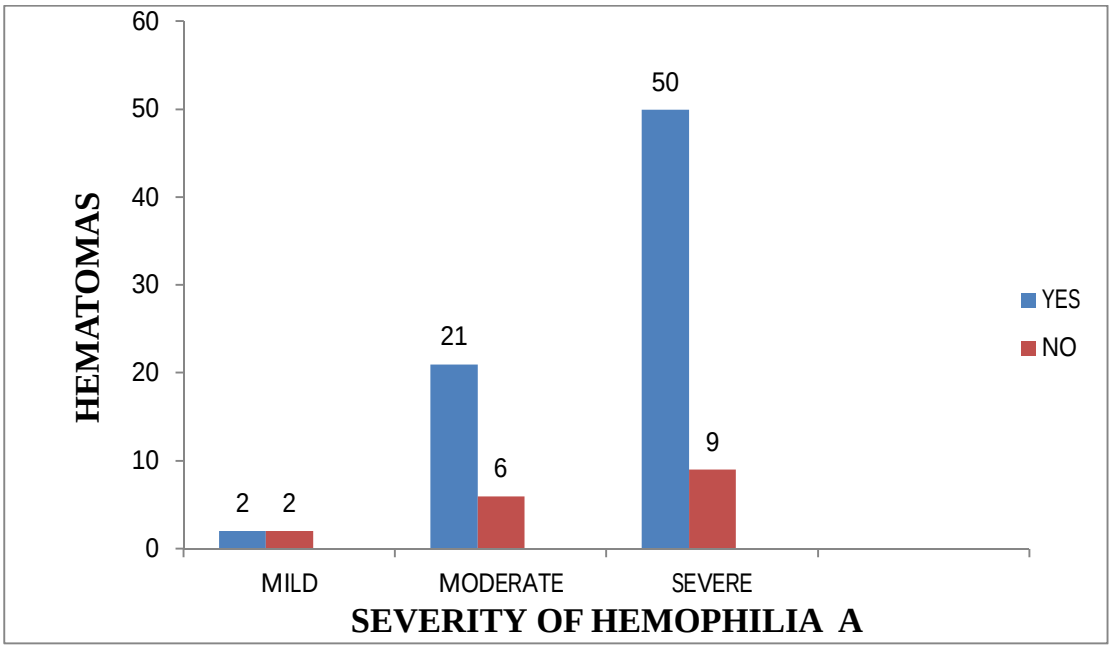


FIGURE.14.HEMATOMAS &SEVERITY OF HAEMOPHILIA A

Among the 59 severe haemophilia A patients, 50 (68.25%) had hematomas, followed by moderate (28.8%) and mild (2.7%) haemophilia A.

Table.12. ASSOCIATION BETWEEN PATIENTS WITH HEMATURIA & SEVERITY OF HAEMOPHILIA A

Hematuria	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	1	2.5%	3	7.5%	36	90.0%	**0.000**
NO	3	6.0%	24	48.0%	23	46.0%	

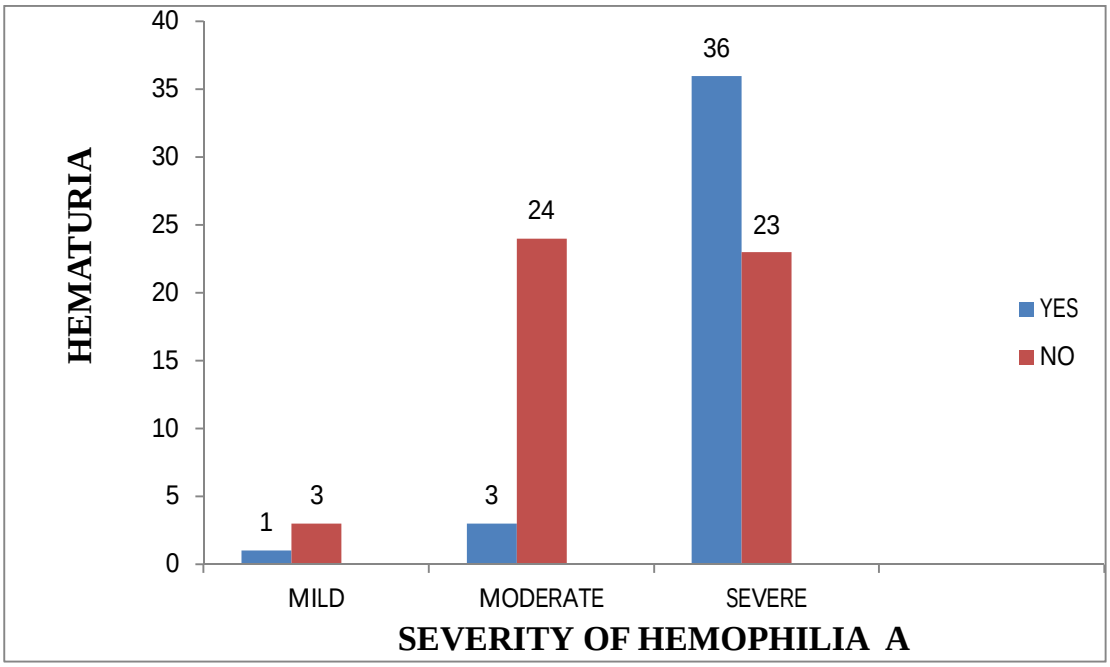


FIGURE.15.HEMATURIA &SEVERITY OF HAEMOPHILIA A

In our study group, hematuria was highest among the severe haemophilia A patients.

This association is statistically significant with **p value of 0.000.**

Table.13. ASSOCIATION BETWEEN PATIENTS WITH UMBILICAL BLEED & SEVERITY OF HAEMOPHILIA A

Umbilical bleed	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	0	0%	0	0%	5	100%	**0.114**
NO	4	4.7%	27	31.8%	54	63.5%	

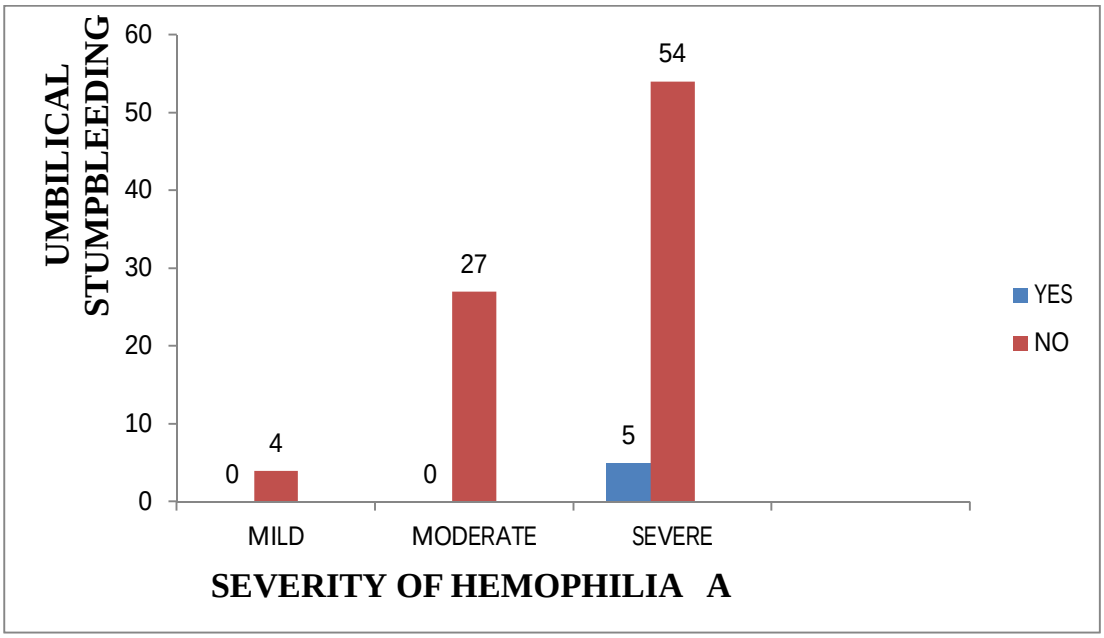


FIGURE.16.UMBLICAL STUMP BLEED &SEVERITY OFHAEMOPHILIA A

Among the 90 haemophilia A patients ,only 5 patients had umbilical stump bleeding. All the 5 patients were diagnosed as severe haemophilia A.

Table.14. ASSOCIATION BETWEEN RETROPERITONEAL BLEEDING & SEVERITY OF HAEMOPHILIA A

Retroperitoneal bleed	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	0	0%	1	5.8%	16	94.1%	0.003
NO	4	5.4%	26	35.6%	43	58.9%	

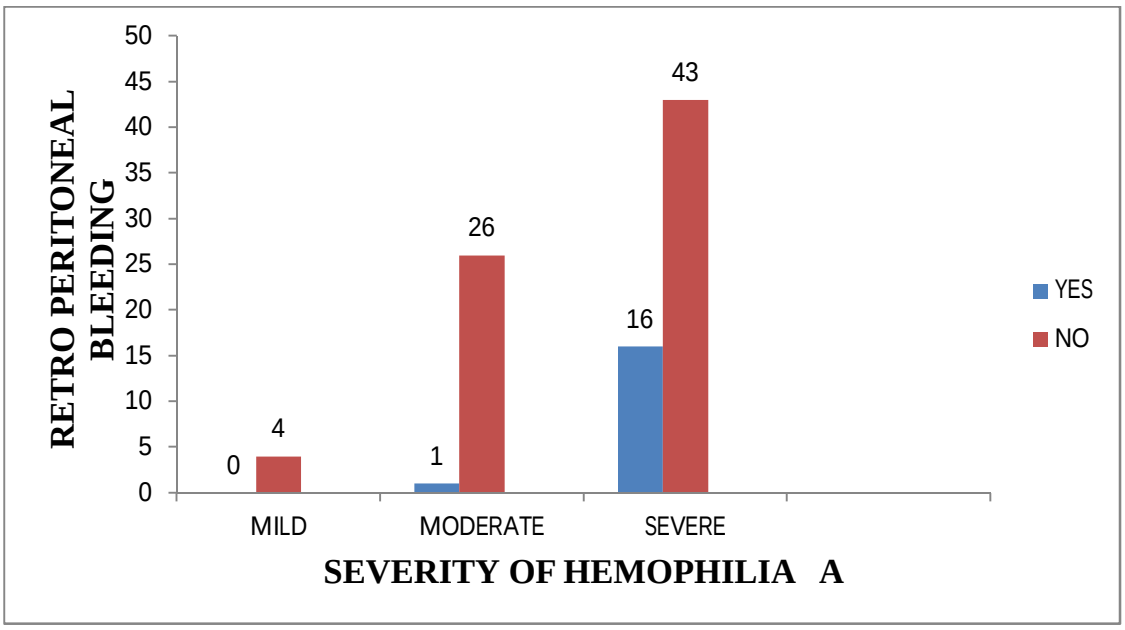


FIGURE.17.RETROPERITONEAL BLEED &SEVERITY OF HAEMOPHILIA A

In our study group, Out of 90 patients 17 patients had retroperitoneal bleed, among 17 patients , 16 were diagnosed as severe haemophilia A.

Table.15. ASSOCIATION BETWEEN PATIENTS WITH INTRA CRANIAL HEMORRHAGE (ICH) & SEVERITY OF HAEMOPHILIA A

ICH	Mild		Moderate		Severe		p value
	Frequency	%	Frequency	%	Frequency	%	
YES	0	0%	0	0%	2	100%	**0.427**
NO	4	4.5%	27	30.7%	57	64.8%	

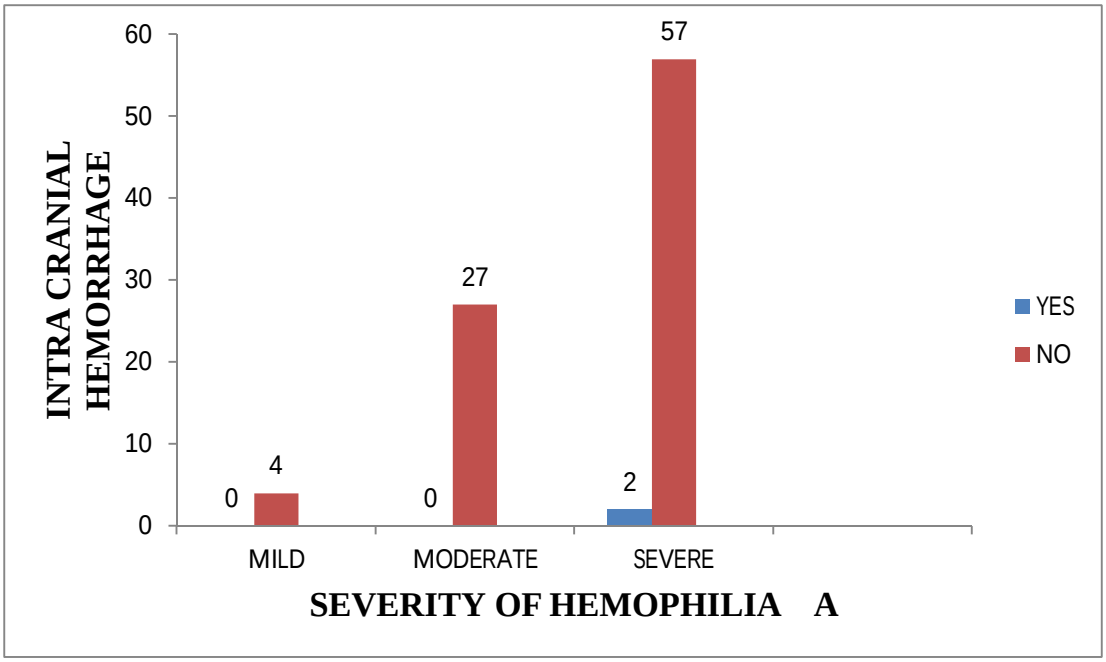


FIGURE.18.INTRA CRANIAL HEMORRHAGE & SEVERITY OF HAEMOPHILIA A

Out of 90 patients, 2 patients had intra cranial hemorrhage.

INHIBITORS IN HAEMOPHILIA A PATIENTS

Table.16. PREVALENCE OF INHIBITORS IN HAEMOPHILIA A PATIENTS

Inhibitor	Frequency	Percentage
Positive	3	3.3%
Negative	87	96.7%

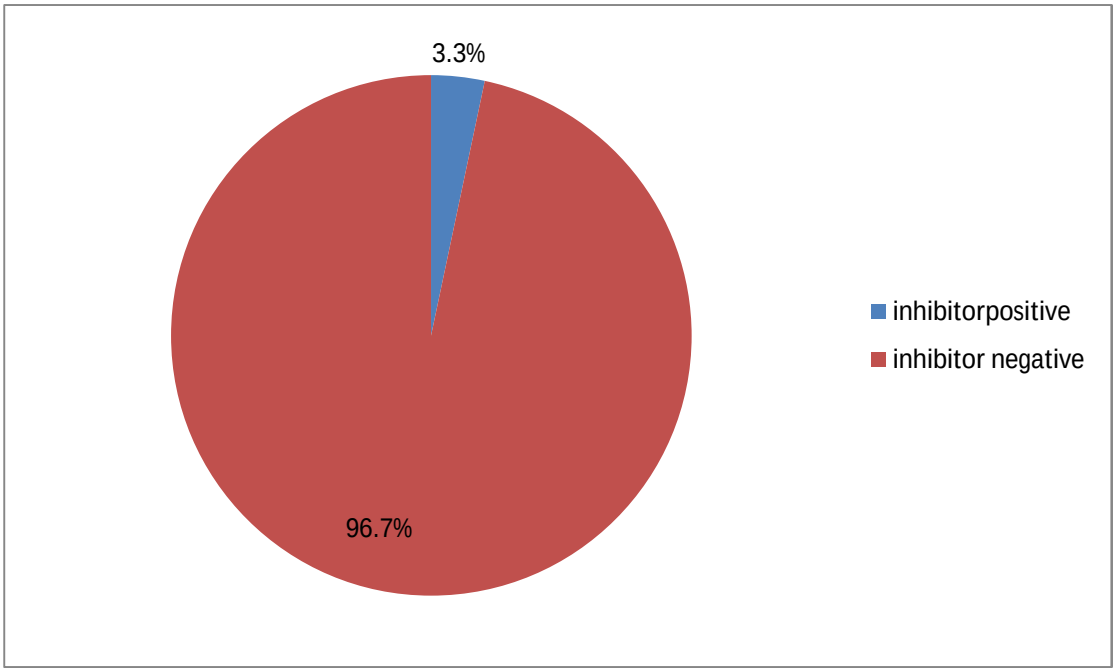


FIGURE .19.PREVALENCE OF INHIBITORS

Out of 90 patients were screened, 3 (3.3%) were found to have developed inhibitors, while 87(96.7%) did not develop inhibitors.

Table.17.PREVALENCE OF LOW AND HIGH TITRE INHIBITORS

Inhibitor	Frequency	Percentage
Negative	87	96.7
High titre	1	1.1
Low titre	2	2.2
Total	**90**	**100**

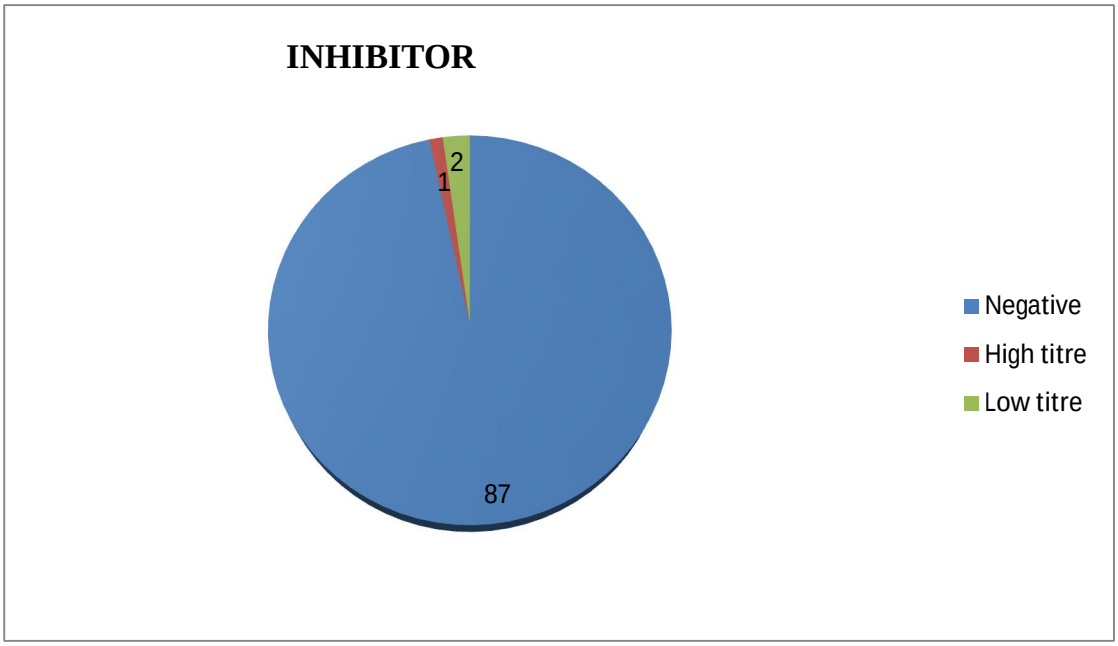


FIGURE.20.LOW TITRE & HIGH TITRE INHIBITOR

Out of 3 (3.3%) patients positive for inhibitor development, one (1.1%) was high titre inhibitor positive and two(2.2%) were low titre inhibitor positive.

Table.18. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT &SEVERITY OF HAEMOPHILIA A

Inhibitor	Mild		Moderate		Severe	
	Frequency	%	Frequency	%	Frequency	%
POSITIVE	0	0%	0	0%	3	5.1%
NEGATIVE	4	100%	27	100%	56	62.2%

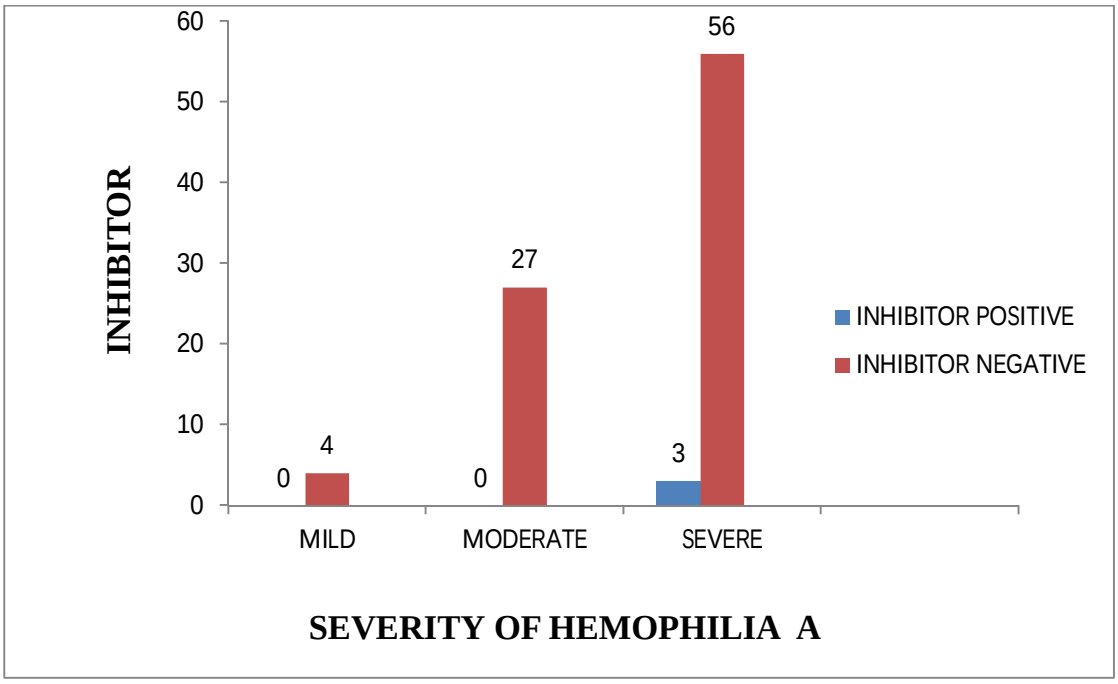


FIGURE.21.INHIBITOR &SEVERITY OF HAEMOPHILI A

All patients who developed inhibitors belonged to Severe haemophilia, which is in accordance with finding that inhibitors develop more commonly in Severe haemophilia group.

Table.19. ASSOCIATION BETWEEN INHIBITORS & FAMILY HISTORY OF HAEMOPHILIA A

Family H/o Haemophilia	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percent	Frequency	Percent	
Positive	3	5.7%	49	94.3%	**0.132**
Negative	0	0.0%	38	100%	

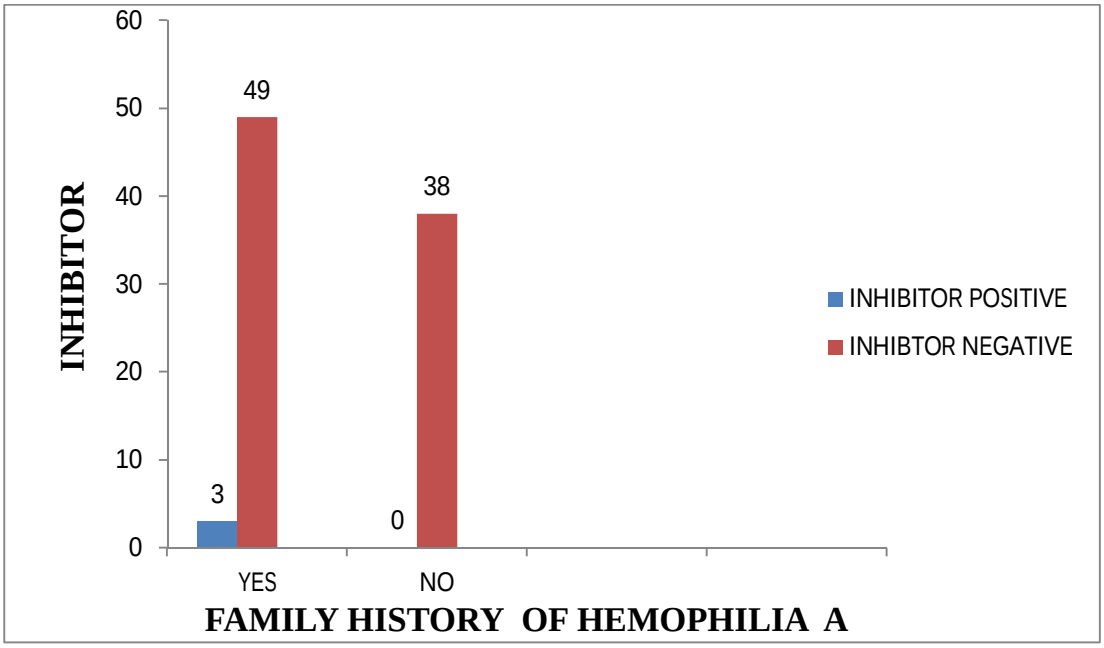


FIGURE.22.INHIBITOR &FAMILY H/O HAEMOPHILIA A

All 3 inhibitor developed patients had positive family history of haemophilia A.

Table.20. ASSOCIATION BETWEEN FAMILY HISTORY OF INHIBITORS & INHIBITOR DEVELOPMENT

Family H/o inhibitors	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	%	Frequency	%	
YES	2	40.0%	3	60.0%	.007
NO	1	1.2%	84	98.8%	

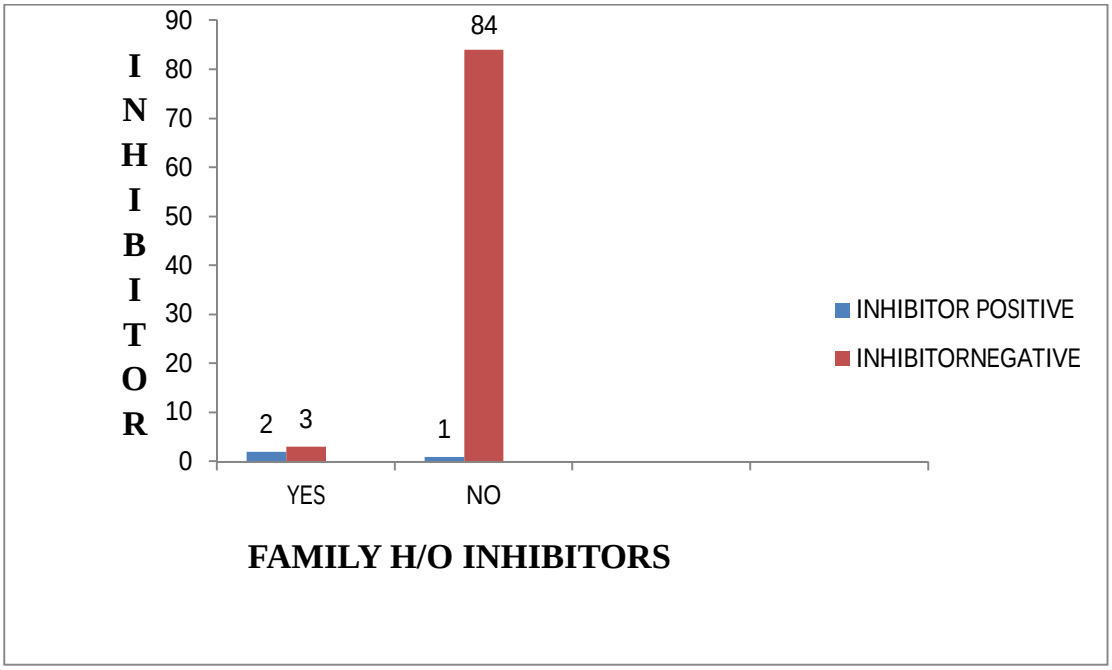


FIGURE.23.INHIBITOR & FAMILY H/O INHIBITOR

Out of 3 patients who developed inhibitors , 2 had positive family h/o inhibitors.

This association was statistically significant with **p vale of 0.007.**

Table.21. ASSOCIATION BETWEEN CONSANGUINITY & INHIBITORS DEVELOPMENT

Consanguinity	Inhibitor positive		Inhibitor negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	1	6.3%	15	93.8%	.474
NO	2	2.7%	72	97.3%	

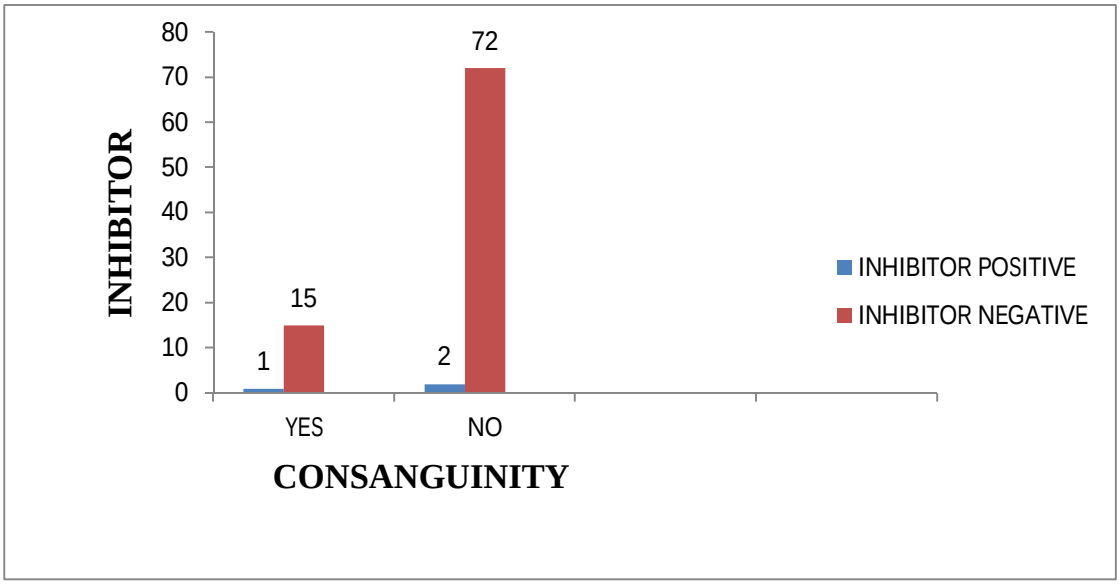


FIGURE.24.INHIBITOR &CONSANGUINITY

Among patients (16) born out of consanguineous marriage, one (6.3%) developed inhibitor.

ASSOCIATION BETWEEN CLINICAL MANIFESTATIONS AND INHIBITOR DEVELOPMENT

Table. 22. ASSOCIATION BETWEEN PATIENTS WITH SPONTANEOUS BLEEDING & INHIBITOR DEVELOPMENT

Spontaneous bleeding	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	3	3.9%	73	96.1%	.455
NO	0	0	14	100%	

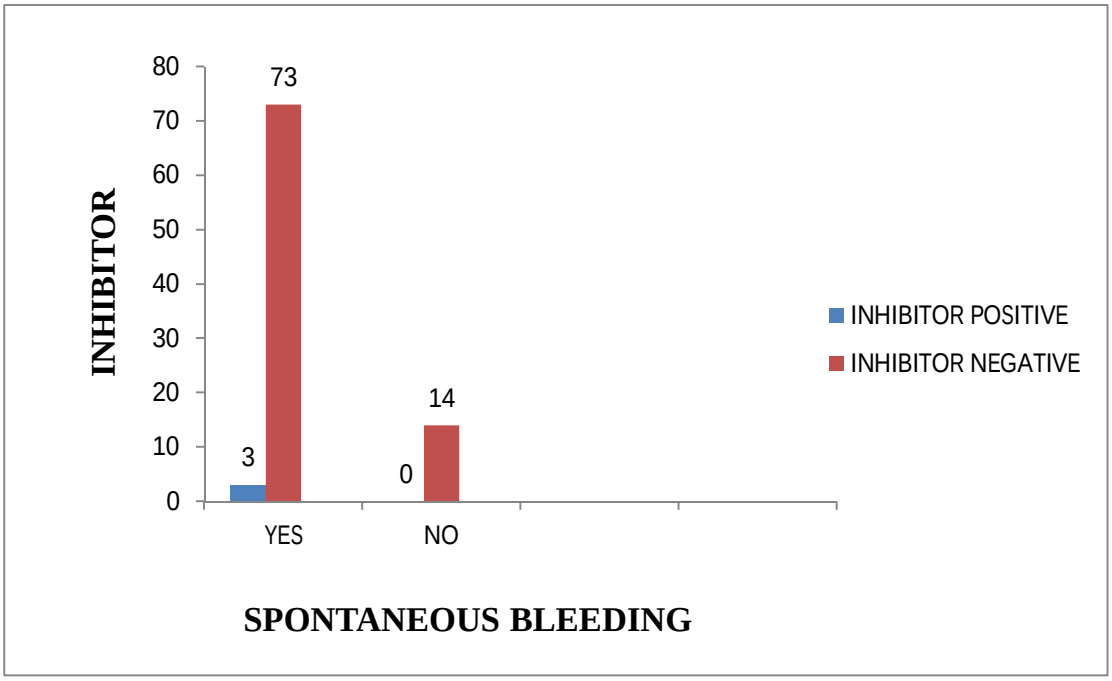


FIGURE.25.INHIBITOR & SPONTANEOUS BLEEDING

All 3 inhibitor positive patients had history of spontaneous bleeding.

Table.23. ASSOCIATION BETWEEN PATIENTS WITH UMBILICAL STUMP BLEEDING & INHIBITOR DEVELOPMENT

Umbilical stump bleed	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	1	20%	4	80%	.033
NO	2	2.4%	83	97.6%	

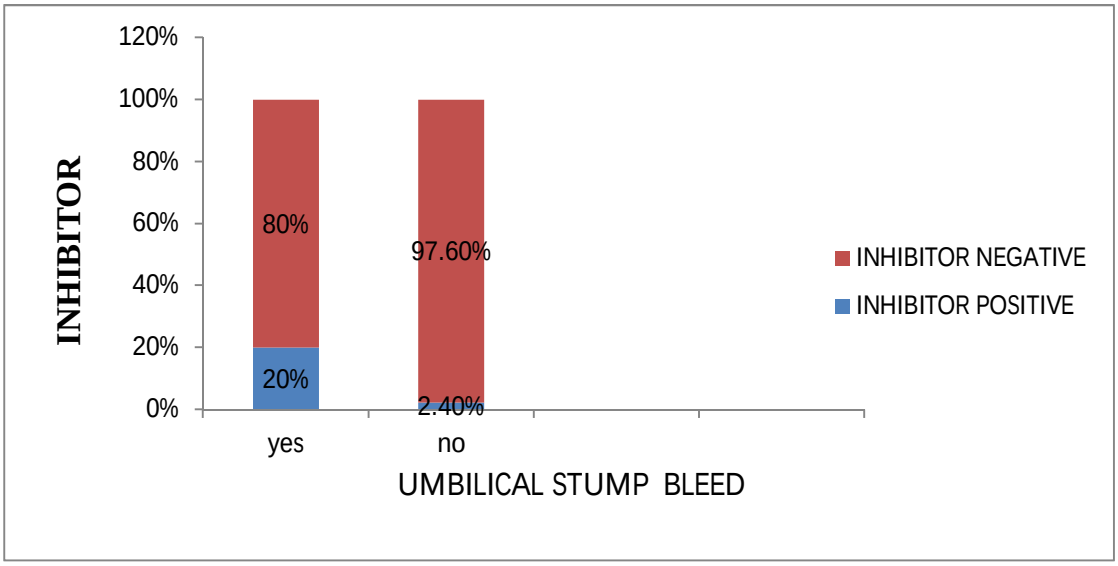


FIGURE .26.INHIBITOR &UMBILICAL STUMP BLEEDING

Among the 90 patients , 5 patients had the history of umbilical bleed, one developed inhibitor.

This association was statistically significant with the **p value of 0.033.**

Table.24. ASSOCIATION BETWEEN PATIENTS WITH HEMATURIA & INHIBITOR DEVELOPMENT

Hematuria	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	3	7.5%	37	92.5%	.050
NO	0	0%	50	100%	

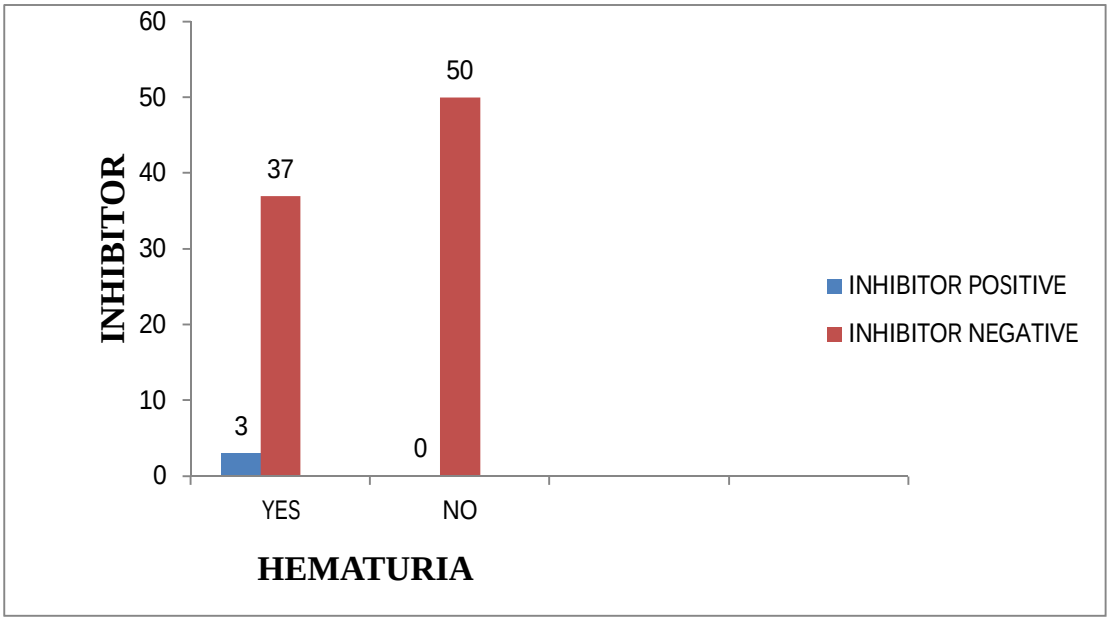


FIGURE.27.INHIBITOR & HAEMATURIA

Out of 90 patients , 40 patients had the history of hematuria, among them 3 patients were developed inhibitor.

This association was statistically significant with the **p value of 0.05.**

Table.25. ASSOCIATION BETWEEN INTRA CRANILA HEMORRHAGE (ICH) & INHIBITOR DEVELOPED PATIENTS :

ICH	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	1	50%	1	50%	0.000*
NO	2	2.3%	86	97.7%	

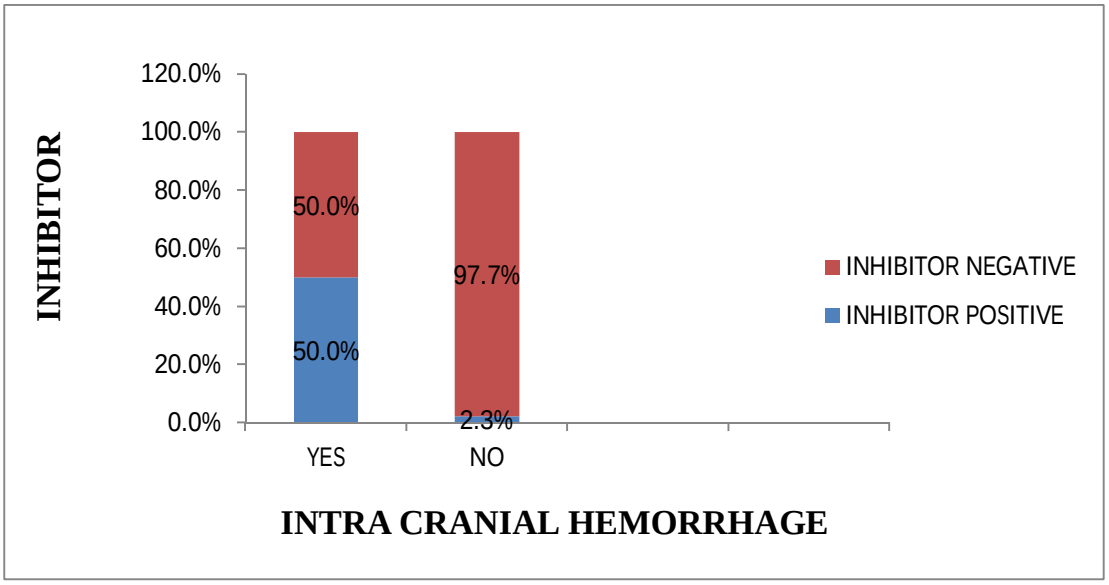


FIGURE.28.IHIBITOR &INTRA CRANIAL HAEMORRHAGE

Among the 90 patients ,2 patients had the history of intra cerebral hemorrhage.1 (50%) patient developed inhibitors.

This association was statistically significant with the **p value of 0.000.**

Table.26. ASSOCIATION BETWEEN CRIPPLING ARTHROPATHY & INHIBITOR DEVELOPED PATIENTS

Crippling arthropathy	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	.033
YES	1	20%	4	80%	
NO	2	2.4%	83	97.6%	

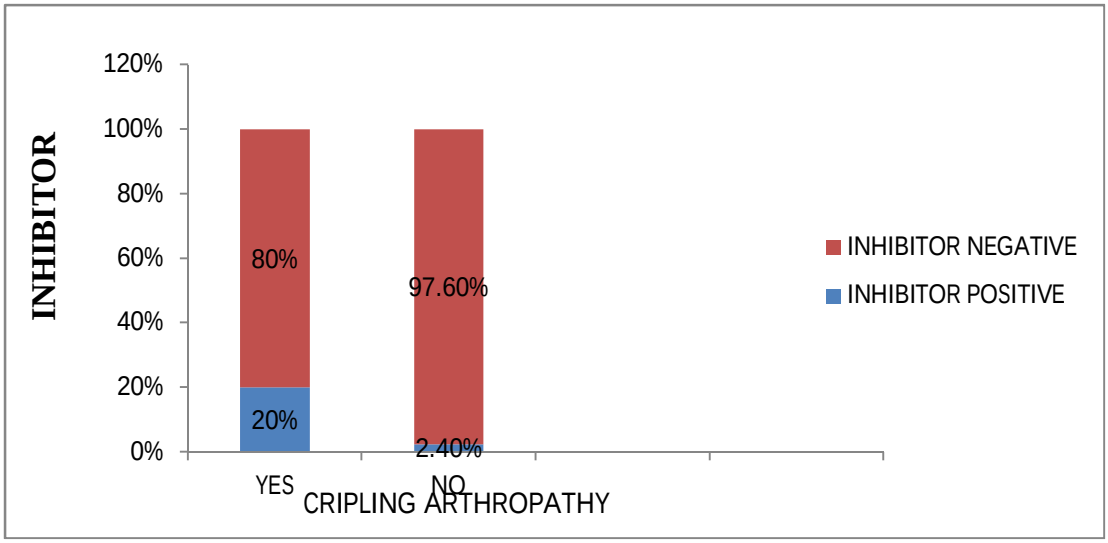


FIGURE.29. INHIBITOR & CRIPPLING ARTHROPATHY

Out of 90 patients,5 patients had the history of crippling arthropathy, among the 5 patients, one patient developed inhibitor.

This association is statistically significant with **p value of 0.033.**

ASSOCIATION BETWEEN TREATMENT RELATEDRISK FACTORS & INHIBITOR

Table.27. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT & AGE AT WHICH FVIII EXPOSURE

Age at which exposed to FVIII	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
<6 months	0	0%	2	100%	.791
>6 months	3	3.4%	85	96.6%	

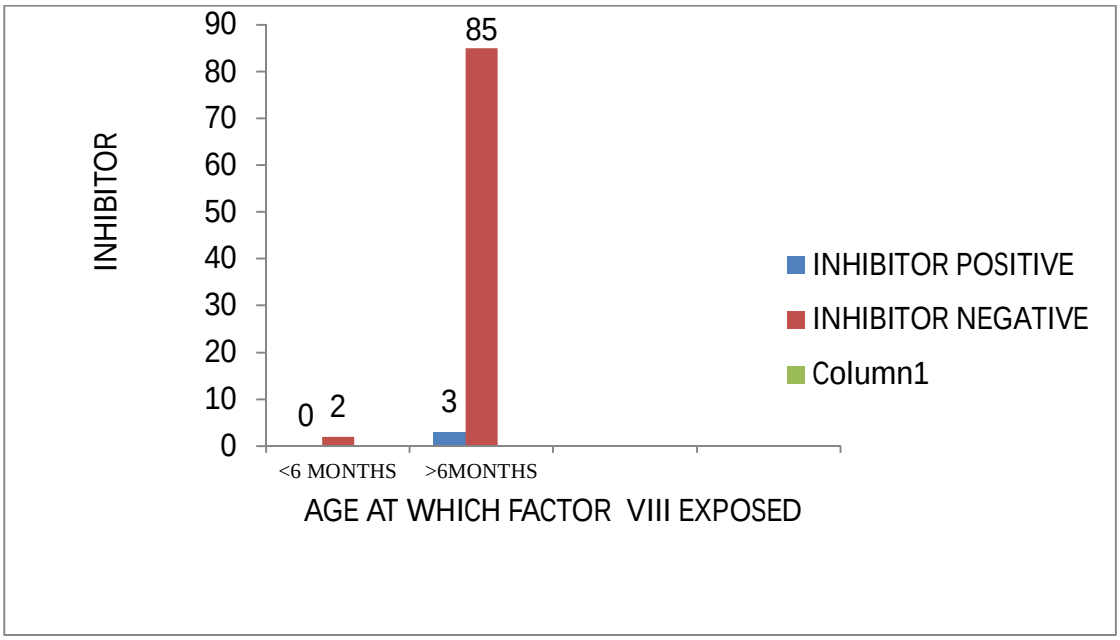


FIGURE.30. ASSSOCIATION BETWEEN INHIBITOR & AGE AT WHICH FACTOR VIII EXPOSED.

Relatively high frequency of inhibitor positivity was found in patients who received their first factor VIII transfusion after the 6 months of age group

Table.28. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT & NUMBER OF FVIII EXPOSURES

No. of times Factor VIII Exposures	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
<50	0	0%	21	100%	.331
>50	3	4.3%	66	95.7%	

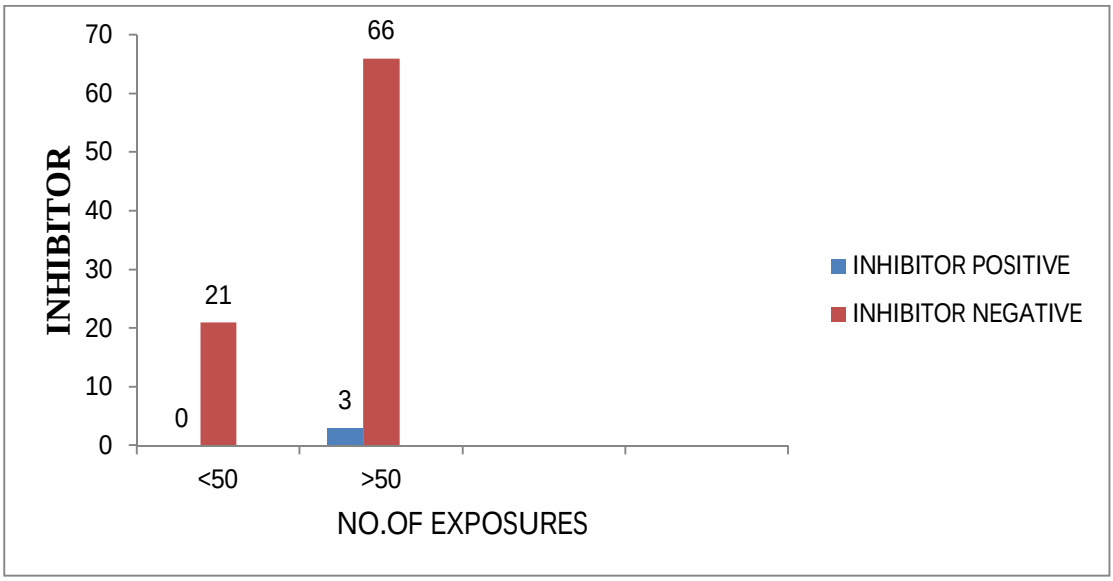


FIGURE.31.ASSOCIATION BETWEEN INHIBITOR &NUMBER OF EXPOSURES TO FACTOR VIII

Inhibitor were seen to develop in patients with exposure to Factor VIII >50 times.

Table.29. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT & DOSE OF FVIII

Dose of FVIII	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
>50IU/Kg	3	50%	3	50%	.000
<50iu/kg	0	0%	84	100%	

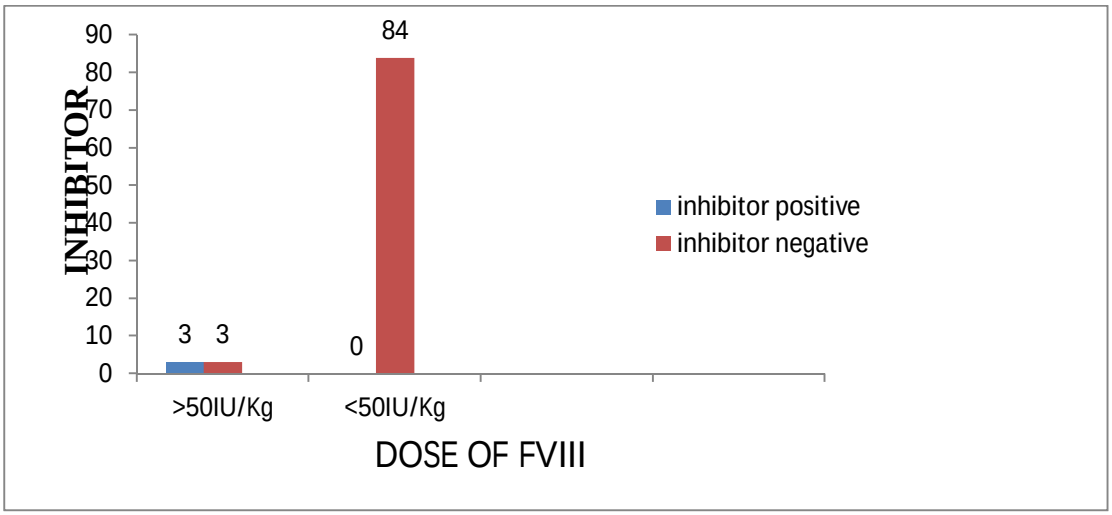


FIGURE.32.ASSOCIATON BETWEEN INHIBITOR & DOSE OF FACTOR VIII

Out of 90 patients , 6 were treated with >50IU/Kg .among the 6 patients, 3 had developed inhibitors.

This association was statistically significant with **p value of 0.000.**

Table.30. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT& INTERVAL BETWEEN EXPOSURE DAYS

Interval between exposure days	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	percentage	Frequency	Percentage	.000
<10	3	75%	1	25%	
>10	0	0%	86	100%	

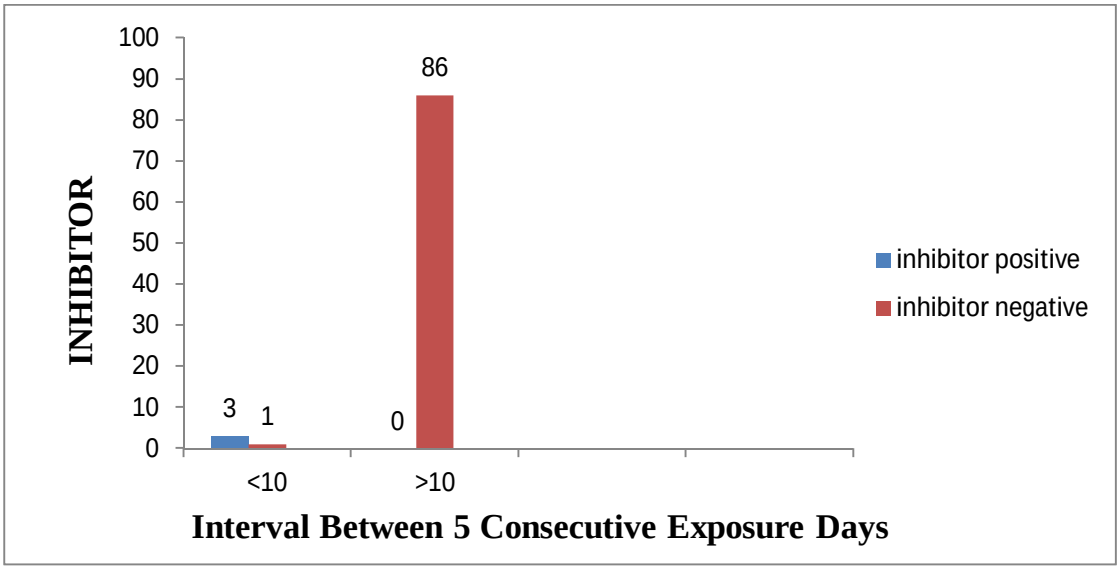


FIGURE.33.ASSOCIATION BETWEEN INHIBITOR &INTERVAL BETWEEN EXPOSURE DAYS

4 patients had the interval between 5 consecutive exposure days is less than 10 days , out of 4 patients 3 had developed inhibitor.

This association was statistically significant with the **p value of 0.000.**

Table.31. ASSOCIATION BETWEEN DEVELOPMENT OF INHIBITORS & SURGERY WITHIN 50 DAYS OF EXPOSURE

Surgery within 50 days of exposure	Inhibitor Positive		Inhibitor Negative		p value 0.159
	Frequency	Percentage	Frequency	Percentage	
YES	1	20%	4	80%	
NO	2	2.4%	83	97.6%	

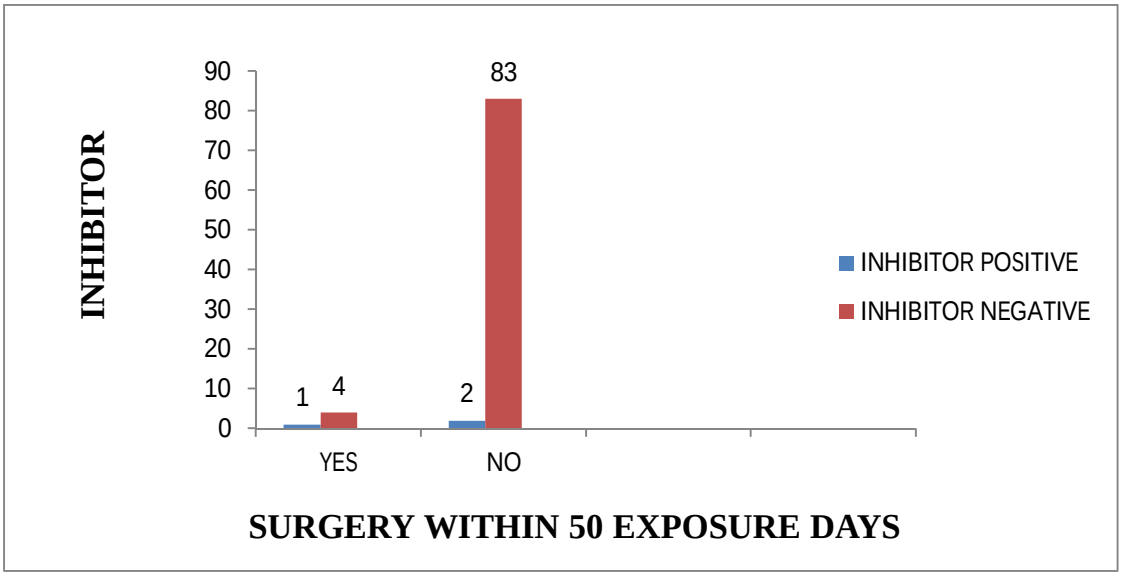


FIGURE.34.ASSOCIATION BETWEEN INHIBITOR &SURGERY WITHIN 50 EXPOSURE DAYS

In 5 patients who had major surgeries within 50 days of exposure, 1 developed inhibitors.

Table.32. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT AND RESPONSE TO TREATMENT

Response to treatment	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	.000
YES	0	0%	83	100%	
NO	3	42.9%	4	57.1%	

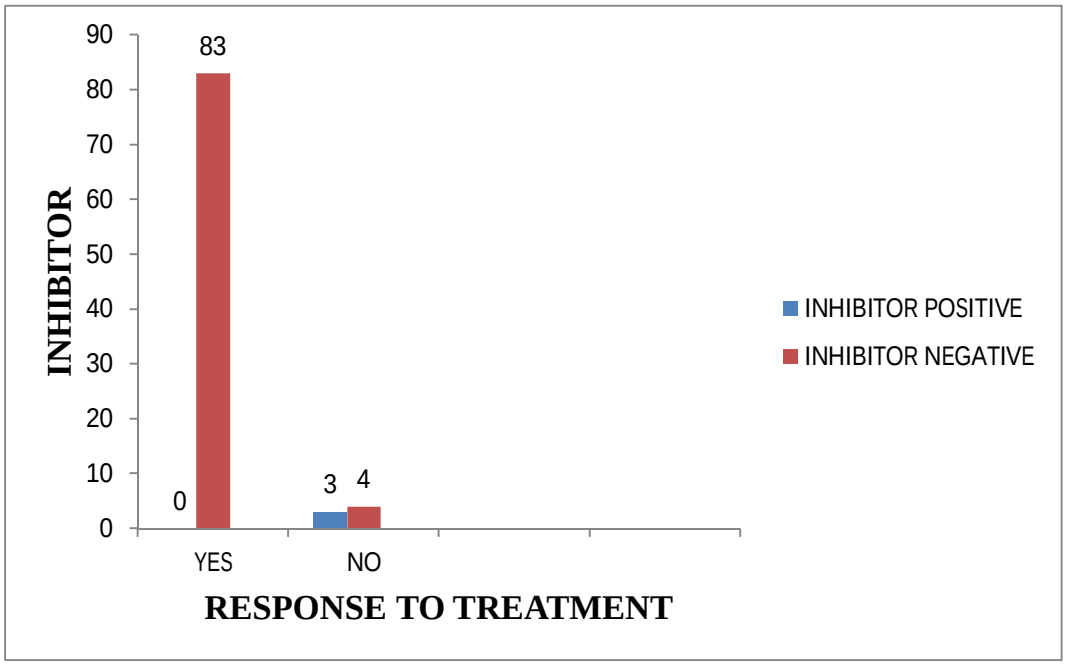


FIGURE.35.ASSOCIATION BETWEEN INHIBITOR & RESPONSE TO TREATMENT

In our study, 7 patients showed poor response to Factor VIII treatment, 3 were developed inhibitors.

This was statistically significant with **p value of 0.000**

Table.33. ASSOCIATION BETWEEN INHIBITOR DEVELOPMENT & FRESH FROZEN PLASMA TRANSFUSION

FFP Transfusion	Inhibitor Positive		Inhibitor Negative		p value
	Frequency	Percentage	Frequency	Percentage	
YES	3	4.5%	64	95.5%	0.408
NO	0	0%	23	100%	

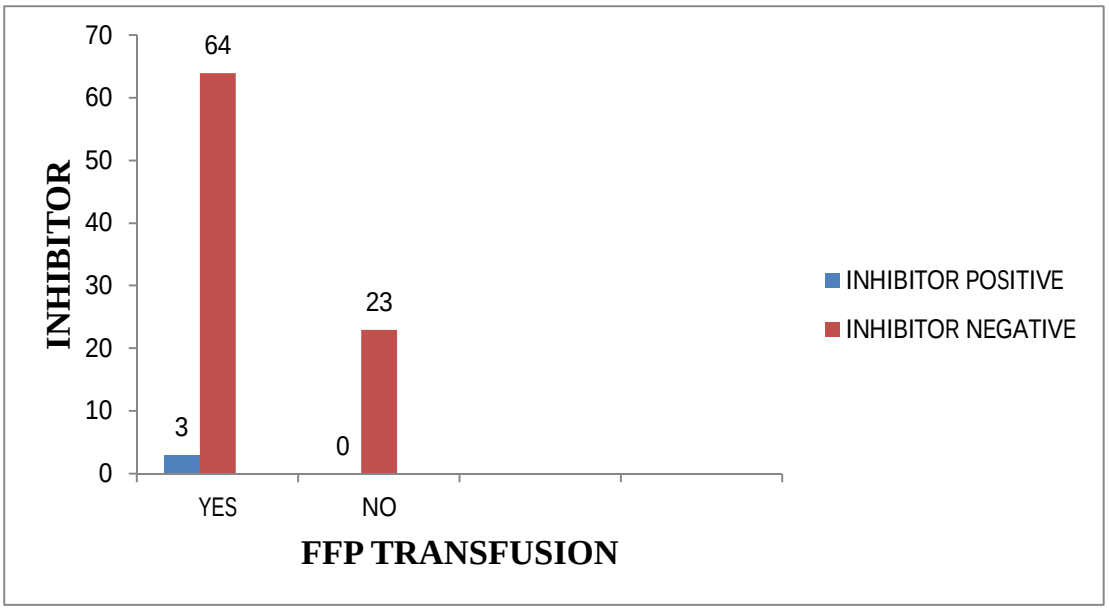


FIGURE.36.ASSOCIATION BETWEEN INHIBITOR &FFP TRANSFUSION

Out of 90 patients, 67 patients had exposure to FFP transfusion, among them 3 patients had developed inhibitors.

DISCUSSION

The development of inhibitors (inhibitory antibodies) against exogenous FVIII remains a most serious complication in the management of haemophilia A .It will make a significant impact on both the morbidity, mortality and three times increase the cost of treatment.[35] The presence of inhibitors has major effects on bleeding control, arthropathy status and quality of life .Inhibitors reduce the efficacy of hemostatic treatment.[49] Management of patients who develop inhibitors is a challenge even in the developed world.[71]

With this background, the aim of our study is to find out the prevalence of inhibitors and analyse the risk factors which are involved in the development of inhibitors.

Several risk factors involved in inhibitor formation including patient related factors which are severity of haemophilia, genetic mutation, HLA genotype, race, ethnicity, and family history of inhibitors. In addition to this, the therapy related factors like, type of FVIII product either recombinant or plasma derived FVIII , age at which first exposure to FVIII therapy, number of exposures, dose of factor VIII given, duration between two consecutive 5 exposure days also plays a role in inhibitor.[78] Another risk factor is intense immunologic stimulation (eg. Surgery) during initial exposure days (<50 days) has the significant correlation with inhibitor development .[59]

Out of 90 patients screened, the age range of patients with haemophilia A was 3-58 years. All were males. In the coagulation profile of haemophilia A patients, mean of APTT was113.5 seconds (range was 48.20 to 240.80).FVIII levels were in the range of <1% - 22%.The patients were categorized as mild ,moderate and severe based on the Factor VIII level. Out of 90 patients, 59(65.6%) were diagnosed as sever hemophiliacs, moderate and mild were 27(30%) and 4(4.4%) respectively .Our study results shows severe>moderate>mild. This finding is supported by Wang et al. In his study, he also reported severe is more common than mild and moderate. his study results shows severe(77.2%) > moderate (17.4%)> mild (5.4%).[72] This findings are in accordance with the finding of soucie et al, in his study about occurrence of haemophilia A in US ,he reported the severe haemophilia A was commoner than mild and moderate.[85]

PREVALENCE OF INHIBITORS:

Out of 90 patients 3(3.3%) were positive on inhibitor screening .Bethesda assay was performed to quantify the inhibitors in these three haemophilia A patients. Out of 3 two were low titre inhibitor (2.2%) one was high titre (1.1%). Our study of 90 haemophilia A patients the prevalence of Factor VIII inhibitor was 3.3%.This findings are similar to the study done by Wang et al for the prevalence of inhibitors in Chinese Haemophilia A patients those who were treated only with plasma derived FVIII, fresh frozen plasma or cryoprecipitate, was 3.9% out of 1435 patients.[72] Another study done in UK by

Wight J et al reported the prevalence of 5-7%.[73] The inhibitor prevalence in our study is similar to the other study done by Ghosh et al in India which is 8.2% out of 352 patients.[63]. Another study in India done by Mathews et al showed the prevalence was 13% out of 200 patients.[71] In a study done by Dubey et al ,the prevalence was 5.1% (n=5)out of 114.[52] The data from all the Indian studies show lower inhibitor prevalence as compared with developed countries.[71] Low prevalence of inhibitors in our patients, may be due to scarce availability of factor concentrates and delayed initiation of factor replacement therapy.[52] Stonebreaker et al , in his study he found out the prevalence of inhibitors in high income countries (12.8±6) was higher than lower income countries(6.6±4.8).Aledort et al described the possible causes for under reporting the cases of hemophilia may be due to lack of diagnostic capability and scarcity of Factor VIII replacement therapy

Inhibitors are classified according to their levels in plasma as a high titre inhibitors ,with the activity ≥5 Bethesda units(BU)/ml or a low titre inhibitor type with the activity of <5 BU/ml.[55] In our study only one patient was found to have high titre inhibitors and the remaining two had low titres .

S.No	Age (yrs)	APTT(Control 28-36 secs)	FVIII%	INHIBITORTITRE BU/ml
1.	25	119.80	<1%	1.3
2.	35	180.00	<1%	4.8
3.	37	180.46	<1%	10

I.PATIENT RELATED RISK FACTORS:

The mean age of patients in the study is 22.95 years. In our study the age of patients ranged from 3 years to 54 years. The mean age of the patient for inhibitor development is 32.3 yrs. Ghosh et al reported the mean age at development of inhibitors was 17.7 years (range 6±52 years).[63] In his study, he found out ,one severe haemophilia A patient was detected inhibitor during his 42 years of age. Mathew et al says while doing the cross sectional study, it was not possible to assess the age at which inhibitors first developed or comment on the duration of their persistence.[70.] Factor VIII inhibitors rise in patients with haemophilia A throughout life with a bimodal risk.[27] As life expectancy increases, the rising incidence of inhibitor development in older patients with severe haemophilia A will become a more important clinical challenge. Kempton et al explains that the older haemophilia A patients have more exposures compared with younger patients, therefore considered to be at a higher risk for inhibitor development. Similar finding was observed from analysis of the United Kingdom Nationwide Database by Hay and colleagues.[74]

Family History of Haemophilia A :

Out of the total 90 patients, half of the patients {52 (57.8%)}, had positive family history. Among the 52, 41 were diagnosed as severe haemophilia A,(p value 0.004) .Further, all three inhibitor positive patients are belonged to severe haemophilia A with positive family history. However the

association between inhibitor development and positive family history is statistically insignificant with the p value of 0 .132.This finding is supported by Gouw et al, he reported the inhibitor risk was similar in patients with positive and negative family history of hemophilia.[59]

FAMILY H/O INHIBITOR:

Former and recent studies on inhibitor development showed that patients with severe haemophilia A and positive family history of inhibitor are at highest risk of developing an inhibitor .[79,80,81,82] In our study out of 3 inhibitor positive patients ,two (40.0%) had positive family history of inhibitors. In our study the association between family history of inhibitor and the inhibitor development is statistically significant with the p value (.007). The risk of developing inhibitors was 3 fold higher in patients with a family history of inhibitors than in patients with a negative family history .[59].Out of inhibitor positive patients 1/3 had the sibling with the h/o inhibitor. Gill et al reported that the risk of inhibitor development in the hemophilic sibling of an inhibitor patient is approximately 50%.

The second possible cause is genetic. Some hemophiliacs with certain type of mutation like large deletion, null mutation, inversion 22, of their F8 gene may be more prone to develop inhibitors. Family members often share the same gene mutation. This explains why development of inhibitors runs in families. [73]

CONSANGUINITY:

Out of 90 patients, 16 patients (17.8%) had the positive history for consanguineous. Out of 3 inhibitor developed patients, one patient (6.3%) had positive history for consanguineous. The results of two independent studies showed, 10 fold higher incidence seen in consanguineous marriage.[19,76] However this finding is statistically insignificant in our study.

SEVERITY OF HAEMOPHILIA:

All patients who developed inhibitors belonged to severe hemophilia which is in accordance with finding that severe hemophilia is a risk factor for inhibitor development.[59] Out of 90 patients, 59(65.6%) patients are belonged to severe hemophilia. Out of 59 patients, 3 (5.1%) were positive for inhibitors. This finding is similar to a study done by Jenny et al in which 11% of individuals with severe haemophilia developed inhibitors following plasma derived factor VIII transfusion. This finding was supported by Wang et al ,in his study the prevalence of inhibitor developed more common in severe(4.3%),then moderate(2.4%)followed by mild(2%).[72.] However, the prevalence of inhibitors in patients with severe haemophilia A reported from developed countries is as high as 30%. Mutation in the F8 gene has a predictive value in the severity of disease will have the influence of development of inhibitors. Among patients with severe hemophilia A, large deletion,intron 22 inversion and stop codon association will have 35% risk of inhibitor formation.[19]

II. TREATMENT RELATED RISK FACTORS:

AGE AT WHICH FIRST EXPOSURE TO FACTOR VIII :

In our study all inhibitor positive patients receive their first dose of Factor VIII in the age group between 10 -20 years of age. This is contrast finding to several studies which showed that most inhibitors develop in children with severe haemophilia ,exposure to FVIII during the first 6 months of life.[42] According to Lorenzo et al the early exposure (< 6 months) to FVIII therapy is the risk factor for inhibitor development.[87] Other various studies, CANAL study and Chalmers study, Bom et al showed the relationship between inhibitor development and treatment characteristics in previously untreated patients with severe haemophilia A and confirmed that an early age of first exposure to FVIII was associated with an increased risk of inhibitor development.[42] However further analysis showed that this association was disappeared after adjustment of dose of FVIII & intensity of exposure.[59] In our study ,the association between inhibitor development and age at first exposure was statistically insignificant (p value .791).This finding was supported by Gouw et al, in his study he explained there was no apparent relation between age at first treatment and risk of developing clinically relevant inhibitors.

NUMBER OF EXPOSURE DAYS:

In CANAL study showed that the highest risk of developing inhibitors was observed within the first 50 exposures to FVIII ,with the risk reducing after 200 treatment days.[45] In our study all the inhibitors developed after the 50 exposure days ,but within 150 exposure day. These patients are not followed up

in a single centre hence the number of exposure days are obtained from the patient history, which may not be infallible. Considering the fact the patients had their inhibitors detected after the 50 exposure days. However this finding was not statistically significant in our study.

DURATION BETWEEN EXPOSURE DAYS:

Reduced duration between exposure days was significantly associated with increased risk of inhibitor development.[45] In our study, 4 patients had the history of this intensive treatment, among the 4 patients 3 had developed inhibitor .this association is statistically significant with the p value of 0.000. Duration between the 5 consecutive exposure days was fewer than 10 days, the relative risk of inhibitor development was 1.9 times higher than the duration between the exposure days was 10 to 50 days.[59] The duration between the two consecutive exposure days was reduced, it indirectly indicates the increased frequency of FVIII exposures with the increased risk of inhibitor formation.[59]

DOSE OF FACTOR VIII:

In our study ,all 3 inhibitor positive patients had been administered the dose of FVIII is >50IU/Kg body ,this association was statistically significant with the p value 0.007.this finding was supported by Gouw et al, he reported that ,the inhibitor development was 3.3 times higher in patient receiving the mean dose of >50IU/kg.[59]

RESPONSE TO TREATMENT:

In our study, all 3 patients positive for inhibitors showed poor response to therapy. This had a statistically significant p value of 0.000.The similar finding was observed by darby et al, in his study he showed the association between poor response to treatment and inhibitor development.[75]

SURGERY DURING INITIAL 50 EXPOSURE DAYS :

Among 3 inhibitor developed patients, one had exposure to surgery at the initial exposure days. Gouw et al reported that the intense stimulation to immune system (surgeries) or any intense replacement therapy within 50days of exposure were associated with an increased risk of developing inhibitors.[59]

FFP/CRYOPRECIPITATE TRANSFUSION :

Out of 90 patients, 67 had been treated with both FFP/cryoprecipitate and plasma derived Factor VIII therapy while remaining 23 patients had exposed only to Factor VIII therapy. All inhibitor developed patients had exposed to both FFP/cryoprecipitate and plasma derived Factor VIII. Initial period, because of the scarcity of factor concentrates they had been treated with FFP and cryoprecipitate, then switch over to plasma derived Factor VIII concentrates. According to kavakali et al the patients been exposed only to FFP showed the lower incidence of inhibitor. This statement is supported by Ghosh et al and oren et al, they have reported the FFP/cryoprecipitate and

whole blood have much less potential to develop inhibitors than highly purified factor concentrates. Gouw et al and Kempton et al reported , switching among the products was not associated with inhibitor formation.[25,36] Out of 67 patients who had exposed to both FFP/cryoprecipitate and plasma derived Factor VIII therapy ,one patient was positive for hepatitis B surface antigen.

Among the clinical manifestations, our study showed the patients with severe haemophilia A had the manifestations of umbilical stump bleeding (p value 0.03), spontaneous bleeding into joints and muscles (0.000) and hematuria with the p value of 0.050 .The complications of inhibitor development, the intra cranial haemorrhage was observed in one patient with the p value of 0.000.

None of our patients had received prophylactic treatment and recombinant FVIII products.

COMPARISON OF OUR STUDY WITH OUTCOME OF OTHER STUDIES

PREVALENCE OF INHIBITOR.

Our study		**OTHER STUDIES**		
Place	Prevalence (No. of patients studied)	Author	Place	Prevalence(No.of patients studied)
Chennai	3.3%(90)	Wight et al	UK	5-7% (1770)
		Rasi et al	FINLAND	17.3% (110)
		Wang et al	CHINA	3.9% (1435)
		Oren et al	TURKEY	5-10% (58)
		Owaidah et al	SAUDI	29.3% (148)
		Ghosh et al	Mumbai	8.2% (352)
		Dubey et al	Lucknow	5.1% (118)
		Mathewset al	CMC Vellore	12% (200)

II.RISK FACTORS FOR INHIBITOR DEVELOPMENT :

RISK FACTORS	Our study	Gouw et al	Wang et al	Santagostino et al	Chambost et al	Chalmers et al	Lorenzo et al	YeeTT	Kreuz et al	Oren et al	Malmo et al	Ghosh et al	Kavakali et al
Severe <1 IU/ml	YES	YES	YES	-	-	-	-	YES	-	YES	YES	YES	YES
Family h/o hemophilia	YES	YES	-	YES	-	YES	-	YES	-	-	-	-	-
Family h/o Inhibitors	YES	YES	YES	YES	-	YES	-	-	YES	-	YES	-	-
Age at which first exposure FVII	NO	YES	-	YES	YES	YES	YES	-	YES	-	-	-	YES
Doseof FVIII>50IU/ml/kg	YES	YES	-	YES	YES	-	-	-	-	-	-	-	-
Reduced Duration between exposure days	YES	YES	-	YES	YES	YES	-	-	-	-	-	-	-
First50 exposure days	NO	YES	-	YES	-	-	-	-	-	-	-	-	YES
Surgerywithinfirst50exposure days	NO	YES	-	-	YES	-	-	-	-	-	-	-	-
FFP less potential	-	-	-	-	-	-	YES	-	-	YES	-	YES	YES

SUMMARY

In our study

- 90 patients who had taken treatment for Haemophilia A were screened for inhibitor assay.
- The age range of the study group was 3 to 58 years.
- All were males.
- Out of 90 patients screened, 59 were diagnosed as severe haemophilia A, 27 were moderate haemophilia A, 4 were mild haemophilia A.
- Out of 59 patients with severe haemophilia 51 were diagnosed before the age of 1 (**p value <0.05**)
- The clinical manifestations of bleeding were significant among severe haemophilia patients. **(p value <0.05**)
- Among 59 patients with severe haemophilia 41 had positive family history. **(p value <0.05)**
- Out of 90 patients, 23 had been treated only by plasma derived factor VIII; the remaining 67 had exposure to FFP and cryoprecipitate in addition to plasma derived factor VIII.
- None of our patients had received recombinant Factor VIII .
- Mode of treatment for all patients was on demand therapy
- 3 out of 90 patients had developed inhibitor against the exogenous Factor VIII.

In this study, we observed the following

RISK FACTORS FOR INHIBITOR DEVELOPMENT:

- All 3 patients who had developed inhibitors belonged to severe haemophilia.

- Among the 3 severe haemophilia patients who had developed inhibitors, 2 had positive family history of inhibitor development. **(p value <0.05)**

- Patients who had administered the Factor VIII dose of >50IU/kg have more risk for inhibitor development. Our study shows significant association with the **p value of <0.05**

- The patients had undergone more intense treatment that is interval between five consecutive exposure days were less than 10 days have more risk for inhibitor development. Our study shows significant association with **p value of <0.05.**

- Patients who did not show clinical response in spite of adequate Factor VIII therapy are at more risk for inhibitor development. Our study shows significant association between poor response to treatment and inhibitor development (**p value < 0.05).**

CONCLUSION

In our study, we observed a significant correlation between the development of inhibitors and modifiable treatment related risk factors such as intense treatment and dose of Factor VIII. The non-modifiable patient related risk factors correlating with inhibitor development were seen in severe haemophilia A patients and with positive family history of development of inhibitors.

The genetically prone patients should be screened regularly for early recognition for the development of Factor VIII inhibitors. In these patients, if the modifiable treatment related risk factors are avoided, Factor VIII refractoriness due to inhibitor development can be delayed.

Further, once the patient had developed inhibitors to Factor VIII concentrates, the cost of care rises exponentially. Hence, it is imperative to adhere to the standard treatment guidelines and delay the development of inhibitors by diligent identification of avoidable risk factors.

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THE TAMIL NADU DR. MGR, MEDICAL UNIVERSITY,
CHENNAI-600032
Institutional Ethics Committee

Proposal No: ECMGR0309054 Date: 19.09.2016

CERTIFICATE

This is to certify that the project **No. ECMGR0309054** entitled **"Prevalence of Factor VIII Inhibitors in Hemophilia A patients who received Factor VIII therapy"** submitted by **Dr. G. Shanthi, DEPARTMENT OF TRANSFUSION MEDICINE** has been approved by the Institutional Ethics Committee, at the meeting held on **15-07-2016,** under the following terms and conditions.

a. This approval is valid for three years or the duration of the project whichever is less from the date of the Certificate.

b. All procedures to be used on participants are professionally acceptable and standardized.

c. All adverse events during the course of study must be recorded and reported to the IEC within a period of seven days

d. Any change in the study procedure/site/investigator should be informed to the IEC.

e. A yearly progress report of the project has to be submitted to the IEC for review.

(Dr. S. Mini Jacob)
Member Secretary
Institutional Ethics Committee
The Tamil Nadu Dr MGR Medical University

INSTITUTIONAL ETHICS COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID. No.06/2016 Meeting held on 14/12/2016
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval **"PREVALENCE OF FACTOR VIII INHIBITORS IN HAEMOPHILIA A PATIENTS WHO RECEIVED FACTOR VIII THERAPY"** submitted by Dr.G.Shanthi., Department of Transfusion Medicine, The Tamil Nadu Dr.M.G.R. Medical University, Chennai-32.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

DEAN
Govt. Kilpauk Medical College,
Chennai-10.

URKUND

Urkund Analysis Result

Analysed Document: PREVALENCE OF FACTOR VIII INHIBITORS IN HAEMOPHILIA A PATIENTS WHO RECEIVED FACTOR VIII THERAPY.docx (D31140670)
Submitted: 10/9/2017 11:36:00 AM
Submitted By: dr_meera_krishnan@yahoo.com
Significance: 1 %

Sources included in the report:

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Instances where selected sources appear:

1

CERTIFICATE –II

This is to certify that this dissertation work titled " **PREVALENCE OF FACTOR VIII INHIBITORS IN HAEMOPHILIA A PATIENTS WHO RECEIVED FACTOR VIII THERAPY"** of the candidate **Dr. G.SHANTHI** with registration number **201531003** for the award of **M.D** in the branch of **XXI (IMMUNOHAEMATOLOGY&BLOODTRANSFUSION).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1 percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

PATIENT INFORMATION SHEET

Inhibitor development in Haemophilia A patients greatly complicates the therapy .Patients with inhibitors experience bleeding that is harder to control once it starts. This study is done to assess the prevalence of inhibitor in haemophilia patients , will help the physician to change the mode of treatment and prevent the complications of inhibitors development

PROCEDURE :

Data will be collected from Patients and Case records

BENEFITS AND RISKS :

If patients developed inhibitors against factor VIII must be informed to the physician, and the mode of treatment will be changed. Minimal risk for patients enrolled in this study.

CONFIDENTIALITY :

Your privacy will be protected in so for as permitted by law. Only your researcher and Ethical committee members will have access to the data collected during the study.

PARTICIPATION :

Your participation in this study is voluntary and you are free to decide now or later whether to continue or discontinue from the study.

NAME OF THE PATIENT :

SIGNATURE :

DATE :

பங்கேற்பாளர்கான தகவல் படிவம்

இரத்தக் கசிவு நோயாளிகளுக்கு, சிகிச்சையின்போது உருவாகும் ஃபாக்டர் 8 க்கு எதிரான ஏதிர்ப்பு சக்தியின் தன்மை குறித்து கண்டறியும் ஆய்வு.

குறிக்கோள்

இரத்தக் கசிவு நோயாளிகளுக்கு, சிகிச்சையின்போது எத்தனை நோயாளிகளுக்கு , செலுத்தபடும் ஃபாக்டர் 8 க்கு எதிராக ஏதிர்ப்பு சக்தி உருவாகிறது என்பதை கண்டறியும் ஆய்வு.

செய்முறை

நோயாளிகளின் மருத்துவமனை குறிப்பேடுகள் மற்றும் நோயாளிகளிடமிருந்து விவரங்கள் பெறப்படும். பின்பு அவர்களின் இரத்தப் பரிசோதனை டாக்டர் எம்.ஜி.ஆர் மருத்துவ பல்கலைகழகத்திலுள்ள குருத்யேற்றுதுறையில் ஆய்வுக்கு உட்படுத்தப்படும்.

பலன்களும் பாதிப்புகளும்

இரத்தக் கசிவு நோயாளிகளுக்கு உருவாகும் எதிர்ப்பு சக்தியின் தன்மை குறித்து ஆராய இந்த ஆய்வு உதவும.இந்த ஆய்வில் நோயாளிக்கு இன்ஹிபிட்டார் இருப்பது தெரிய வந்தால் மருத்துவர்க்கு தெரிவிக்கப்படும்.இதனால் நோயாளியின் சிகிச்சி முறை மாற்றப்படும் .இதன் மூலம் நோயாளிக்கு மிகச் சிறிய அளவிலான பாதிப்புகள் வரலாம்

இரகசிய பாதுகாப்பு

சட்டவரைமுறைறயின்படி தங்களின் சொந்த விசயங்கள் பாதுகாக்கப்படும் .தங்களின் ஆராய்ச்சியாளர் மட்ட இந்த ஆராய்ச்சியின்போது கிடைக்கும் புள்ளி விவரங்ககளை பயன்பயன்படுத்த இயலும்

பங்களிப்பு

இந்த ஆராய்ச்சியில் தங்களின் பங்களிப்பு தன்னார்வமானது.இந்த ஆராய்ச்சியில் தங்களின் பங்களிப்பினைத் தொடர்வதற்கும்,விடுபடுவதற்கும் எந்த நேரமும்தங்களுக்கு உரிமையுன்டு .

நோயாளியின் பெயர்

கையொப்பம்

தேதி

CONSENT

I confirm that I read and understood the information about the above research study dated _____ and I received chance to ask the questions.

My participation in this study is voluntary and I know that I am free to withdraw from the study at any time, without giving any reason and without affecting of my legal rights.

I agree to this access. I know that my identification will not be revealed in any details that is released to third persons or published.

I agree not to restrict or interfere with any data or results that are obtained from this study. I agree to participate in this research study for the above listed purpose.

Patient's name :

Signature : Date :

Patient IP Number :

Signature of the person

who obtains consent : Date :

ஒப்புதல் படிவம்

ஆய்வாளர் சொன்ன _____ நாளது செய்முறை ஆய்வு குறித்த தகவல்களை நான் படித்து அறிந்து புரிந்து கொண்டேன்,என இதன்மூலம் உறுதியளிக்கிறேன். இது குறித்து கேள்விகள் கேட்பதற்க்கும் எனக்கு வாய்ப்பு அளிக்கப்பட்டதையும் உறுதி செய்கிறேன்.

இந்த ஆய்வில் என் பங்கேற்பு முற்றிலும் என் விருப்பம் சார்ந்தது என்பதையும் அறிந்து கொண்டேன்.எந்த காரணமும் குறிப்பிடாமல் எனது சட்ட உரிமை பாதிக்கபடாதவண்ணம் இந்த ஆய்விலிருந்து எப்பொழுது வேண்டுமானலும் விலகிக்கொள்ள எனக்கு உரிமைஉண்டு என்பதையும் அறிந்து கொண்டேண்

இந்தசெயல்முறை ஆய்வுக்கு நான் ஒத்துழைப்பு நல்குகிறேன் என்று வாக்களிக்கிறேன். இந்த ஆய்வுத்தகவல்கள்,மூன்றாவது நபர்களுக்கோ அல்லது விளம்பரத்திற்காக வெளியிடப்படும் போதோ,எனது அடையாளம் அல்லது தனித்துவம் தெரிவிக்கப்படமாட்டாது என்பதையும் நான் அறிந்து கொண்டேன்.

இந்த ஆய்வின் மூலம் பெறப்படும் யாதொறுதகவல் அல்லது முடிவுகளைத் தடைசெய்யவோ அல்லதுகுறுக்கிடவோ மாட்டேன் என்று உறுதி அளிக்கிறேன் .மேற்கூறிய குறிக்கோளை அடைய எடுத்துக் கொள்ளும் இந்தசெய்முறை ஆய்வில் பங்கேற்க நான் முழுமனதுடன் சுயநினைவுடன் சம்மதிக்கிறேன்

நோயாளின் பெயர்

கையொப்பம் தேதி

ஒப்புதல் பெறுபவரின் கையொப்பம் தேதி

PROFORMA

Name of the patient : In Patient Number :

Age /Sex : Blood group :

Diagnosis : Age of Diagnosis :

Clinical manifestations :

Hemarthrosis : Yes/No Most common joint affected:

Haematomas : Yes/No Muscle involvement :

Spontaneous bleeding : Yes/No

Frequency of bleeding episodes /year :

Gum bleeding :

Epistaxis :

Retroperitoneal bleed :

Hematuria :

Umbilical stump bleed :

Intracranial haemorrhage :

Crippling arthropathy :

Family h/o haemophilia : Yes/No

Family h/o inhibitor : Yes/No

H/o consanguinity :Yes/No

H/o surgery :

Whether Mother had bleeding manifestations :

Siblings have the history of haemophilia :

Treatment History ;

Factor VIII Infusion :

Age at first exposure :

Number of infusions

Regular prophylaxis/on demand:

Response to therapy:

Date of last infusion :

Dose of Factor VIII concentrate/infusion :

Interval between the two infusions :

Details of other blood component Transfusion

FFP ,Cryoprecipitate, :

Number of Transfusions