A study of variations of Bleeding time and Clotting time among ABO blood group types and Secretor status in Undergraduate students of Government Medical College(RIMS),Kadapa.

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Abstract

Blood groups plays a vital role in the field of medicine. There is a clear association between ABO blood group types, Secretor status and von Willebrand factor. Deficiency of vWF leads to Hemorrhagic disorders, while elevated levels are a risk factor for thrombosis. Earlier studies state that the O group individuals have prolonged bleeding time and clotting time. Very few studies were done correlating the variations of bleeding time and clotting time and secretor status and von Willebrand factor. The objective of this study was to assess the relationship between Bleeding time and clotting time among various ABO Blood group types and secretor status and also to identify any gender difference among the different ABO blood group types. This is a cross sectional study including 150 Undergraduate students of age group 17 to 20 years. Bleeding time (by Duke's filter paper method) ,Clotting time (by Wright's capillary tube method) and secretor status (by weiner Indirect Haemagglutination test) was determined after obtaining an informed consent from the students. In our study, bleeding time was prolonged among O group secretor and have thinnest blood and are at risk for bleeding tendencies like epistaxis and clotting time less than 4 minutes is more in B group secretors and are at risk for thrombotic diseases like coronary artery disease and atherothrombotic diseases. Gender wise bleeding time and clotting time were higher in females than in males.

Key words: Blood groups, Bleeding time, Clotting time, Secretor status, von Willebrand factor.

Background:

Blood groups play a vital role in the field of medicine. There are many different types of blood groups and ABO blood group system is of great clinical importance. At the turn of 20th century, Karl Landsteiner first described the existence of serological differences between individuals and stated that people of the world, irrespective of their race can be divided into four groups depending on the substances present on the surface of their red blood cells. In 1901, he grouped the individuals into A, B, AB and O. The discovery of the iso-agglutinogens was a milestone in the field of medicine. Karl Landsteiner received the Nobel prize for his discovery of the ABO system of blood groups. [(1)]

The A, B and O genes all locate together at 9q34.1 - q34.2. The genes of the ABO system do not encode directly for the antigens, but encode for enzymes that add specific sugars to the red cell membrane. These sugars are the ABO red cell antigens that are detectable with serological testing. The A gene codes for the transferase α(1,2) N-acetylgalactosamine transferase and the B gene codes for transferase α(1,2) galactosyl transferase and O allele encodes for non-functional transferase.[(3)]

The ABH antigens are found not only on red cells, but also on other cells in the most body fluids and on the cell membranes of tissues such as intestine, urothelium and vascular endothelium. The expression of ABH antigens into body fluids is controlled by the Sese genes and they are located on chromosome 19q13.3.[(2)]

There is some evidence that ABO blood groups may be associated with certain diseases. Certain diseases are more common in individuals with one blood type or the other. Various research data showed the association of diseases like duodenal ulcer, diabetes mellitus, gastric carcinoma, urinary tract infection and venous thrombosis with ABO blood group system.[(4-8)] Gastric cancer has been reported to be more prevalent in

individuals with blood group A, but peptic ulcer is more often seen in those with blood group O.[9] Research groups have found that in O blood group epistaxis is more common as compared to other ABO blood groups, may be due to lower expression of von Willebrand factor (vWF) in them.[10]

The walls of blood vessels and platelets contain vWF, that helps in platelet adhesion and platelet aggregation, and also it regulates circulating levels of factor VIII (anti-hemophilic factor A).[11] vWF plays an important role in temporary hemostatic plug formation and activate clotting mechanism that leads to definite clot formation. Hemorrhagic disorders are due to deficiency of vWF while its elevated levels are a risk factor for thrombosis. [12-14] Some researchers stated that other genes like gene locus of ABO blood group on the chromosome 9q34 have influence on the vWF gene.[15]

Therefore, the ABO blood group system influences the bleeding time(BT) and clotting time (CT) of an individual. BT is the time interval between the skin puncture and spontaneous unassisted stoppage of bleeding. It is mainly the test for assessment of platelet function[16,17]. CT is the time interval between the entry of blood into the glass capillary tube and formation of fibrin threads.[16]

The relationships between BT, CT and blood groups have influence on certain clinical conditions such as epistaxis, cardiac surgery or thrombosis and many more. According to Mourner AE et al and Qureshi M A et al., there is a clear association between ABO blood group status and von Willebrand factor[18,19].von Willebrand factor is a large glycoprotein produced by endothelial cells and megakaryocytes. Its major function is Hemostasis. Deficiency of vWF leads to hemorrhagic disorders, while elevated levels are a risk factor for thrombosis.[20,21,22]

Thus this study was proposed to find out the relationship of blood group with BT and CT in young adults.

The term ABH secretor refers to secretion of ABO blood group antigens in fluids such as saliva, sweat, tears, semen and serum. Approximately, 80% of people are secretors (SeSe or Sese). People who do not secrete their blood type antigen in their secretions are termed non-secretors. About 15% of the population are non-secretors. For example-

- O Group secrete H antigens.
- A group secrete A and H antigens.
- B group secrete B and H antigens.
- AB group secrete A, B and H antigens.[23]

Similar to the ABO blood types it appears additional genetic information must be linked to the secretor gene, as predictable trends in non-blood type aspects of physiology have a close association with Secretor/Non-secretor status. Aspects of Physiology such as the relative activity of an enzyme called intestinal alkaline phosphatase; propensities toward clotting, reliability of some tumor markers and generalised performance of immune system have predictable trends depending upon your secretor status.[24]

The blood type impacts the clotting ability to a significant degree. Infact, it has been estimated that a significant fraction (30%) of the genetically determined variance in plasma concentration of the

vonWillebrand factor (vWF) clotting factor is directly related to ABO blood type. As a rule, it is blood group O individuals who have the lowest amount of clotting factor.[24]

Secretors have the slowest clotting while Non-secretors have shorter bleeding times and a tendency towards higher levels of the clotting factor VIII and vWF. ABO and Secretor genetics actually further interact to influence blood viscosity. In essence this means that an Blood Type A Non-Secretor will be at the far end of the spectrum with the slowest bleeding times, thickest blood viscosity and the most probability to have high platelet aggregation. On the other end on the continuum will be Blood type O Secretors, who will have longest bleeding time, thinnest blood, and least tendency for platelet aggregation. Because of this, Non-secretors (especially type A's) tend to be at the highest risk for future atherthrombotic and heart disease.[24]

Materials and Methods:

Study Design/ Method of Study:

This is a cross sectional study, which is a type of observational study conducted in the Department of Physiology, Government Medical College, Kadapa. The study was approved by the Institutional Ethical Committee.

Method of Sampling:

150 healthy MBBS first year students among the age group 17-20 years were selected for the study after obtaining the consent. This study was conducted between August 2018 and January 2019in Government Medical College, Kadapa, Andhra Pradesh state, India. Case sheets were filled for the subjects to obtain their medical history and socio-demographic parameters (Age, sex, educational status, occupation, blood groups and willingness to participate in the study). The sample size required was taken for convenience.

A total of 150 subjects were recruited for this study from the first year MBBS Stuents. Both males and females of ages ranging from 17 to 20 years were recruited. Among 150 students,70 were males and 80 were females.

Blood Group Determination:

Blood group was determined by slide agglutination technique.

Principle:

The surface of the red cell membrane contains genetically determined antigens called agglutinogens, while plasma contains antibodies called agglutinins. To determine the blood group of a person, his/ her red cells are made to react with sera containing agglutinins. The slide is then examined under a microscope to detect the presence or absence of clumping and haemolysis of red cells that occurs as a result of antigen-antibody reaction.

Materials :

Antisera, slide, lancet, compound microscope.

Procedure:

Under aseptic precautions, the pulp of the ring finger was pricked by a sterile lancet and one drop of anti-A was placed on one side of a microscopic slide and labelled as A. One drop of anti-B was placed on the

other side of the same slide and labelled as B. A drop of blood was added to each drop of antiserum. Blood groups were determined as follows-

- Agglutination in slide A- Blood Group A
- Agglutination in slide B- Blood Group B
- Agglutination in both slides- Blood Group AB
- Agglutination in neither of the slides- Blood Group O

Determination of Secretor Status:

It is determined by Haemagglutination inhibition technique.(Weiner's test)

Principle:

If the blood group antigens are present in saliva when an appropriate antiserum is added to it, an antigen-antibody reaction occurs. The antibodies in the serum neutralise the antigens in saliva. When a red cell suspension of the same blood group is now added to this mixture, there is no agglutination due to previous inhibition of the antiserum. Thus, in the case of secretors there will be no agglutination seen. In the case of non-secretors, as their saliva does not contain blood group antigens, the antiserum added will not be inhibited by the antigens. Now when the red cell suspension of the same blood group is added to the mixture and there will be an agglutination reaction. Thus, in case of non-secretors, there will be agglutination.

Materials:

1. 5% red cell suspension.
2. Antiserum (Anti-A, Anti-B, Anti-H)
3. Diluted and processed saliva.
4. Test tube rack with tubes.
5. Pipette.
6. Microscope.
7. Slide.
8. Centrifuge.
9. Hot water bath.
10. Sterile containers.

Procedure:

Saliva was collected at room temperature between 11 a.m. and 12 noon and tests were carried out between 12 noon and 4 p.m.

a) Preparation of 5% Red Cell Suspension:

The freshly collected blood in an EDTA bottle was transferred into a small glass tube and centrifuged at a speed of 3000 rpm for 15 minutes to pack the red cells. The supernatant plasma was separated as much as possible from the cells and replaced by sterile isotonic saline. This mixture was centrifuged and again the supernatant separated from the cells. This procedure was repeated 3 to 4 times to wash the red blood cells. Washing of the red blood cells was done to remove any antibody present on the cells. After the last washing, the

supernatant was removed and one drop of packed red cells was mixed with 19 drops of normal saline to prepare a 5% red cell suspension in isotonic saline.

b) Collection and Processing of Saliva:

Saliva was collected at room temperature in sterile disposable containers. The patient was instructed to wash the mouth and drink a glass of water. One millilitre of saliva was collected in a sterile plastic bottle and transferred to a sterile test tube. The tube was then kept in a boiling water bath (100^{o}c) for twenty minutes. Saliva was boiled to destroy the enzymes present. The saliva was then centrifuged at 3000 revolutions per minute (rpm) for fifteen minutes. The supernatant was separated and diluted in a 1: 4 ratios. All the saliva samples were tested for secretor status on the same day of their collection.

c) Dilution of Anti-Serum:

Anti-A and Anti-B antisera were used for patients of Blood Group A and Blood Group B and Anti-H antisera was used for patients belonging to Blood Group O. Antisera were diluted in a dilution of 1: 8. E.g. if diluted anti-A was to be prepared, four test tubes were kept in a row and one drop of saline was put into each tube. One drop of Anti-A was now added to the first tube and mixed. Then one drop of diluted serum was taken from the first tube and added to the second tube. The same procedure was followed up to the fourth tube. (Ref) as shown in the table below:

Tube	Dilution
Tube 1	1:1
Tube 2	1:2
Tube 3	1:4
Tube 4	1:8

One drop of processed saliva and one drop of diluted antiserum from test tube four (1: 8 dilution) were now added to fresh test tube.

d) Procedure to determine Secretor Status:

The contents were mixed well and allowed to stand for fifteen minutes. Then a drop of standard red cell suspension was added and allowed to stand for sixty minutes. The mixed solution was examined under microscope. Care was taken to wash the pipette repeatedly, immediately after each dip. No agglutination suggested the presence of antigen in saliva, while agglutination suggested the absence of antigens in the saliva. Saline controls were kept simultaneously with the test.

Statistical Analysis:

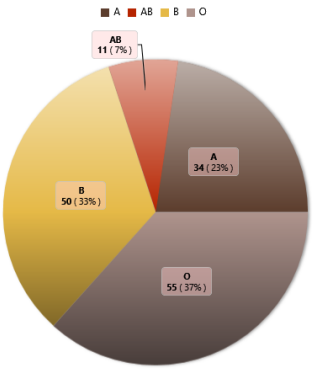
Statistical analysis was done by using EpiInfo app. The Chi-square analysis and Fishers exact was applied to examine relation between ABO blood group types and secretor status and BT and CT; p value of less than 0.05 is considered to be statiscally significant.

Results:

The data of 150 students were collected and analyzed statistically. The study group's age was homogenous (17to 20 years) as everyone belonged to First year MBBS. Out of 150 students there were 80 females and 70 males.

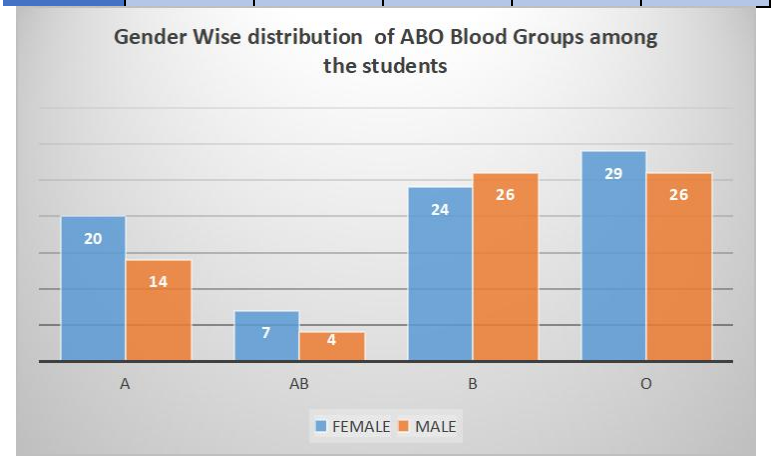
1.Percentage distribution of ABO Blood Group Types among the students:

TYPE OF BLOOD GROUP	FREQUENCY	PERCENT
A	34	22.67%
AB	11	7.33%
B	50	33.33%
O	55	36.67%
	150	100%



2.Gender Wise distribution of ABO Blood Groups among the students:

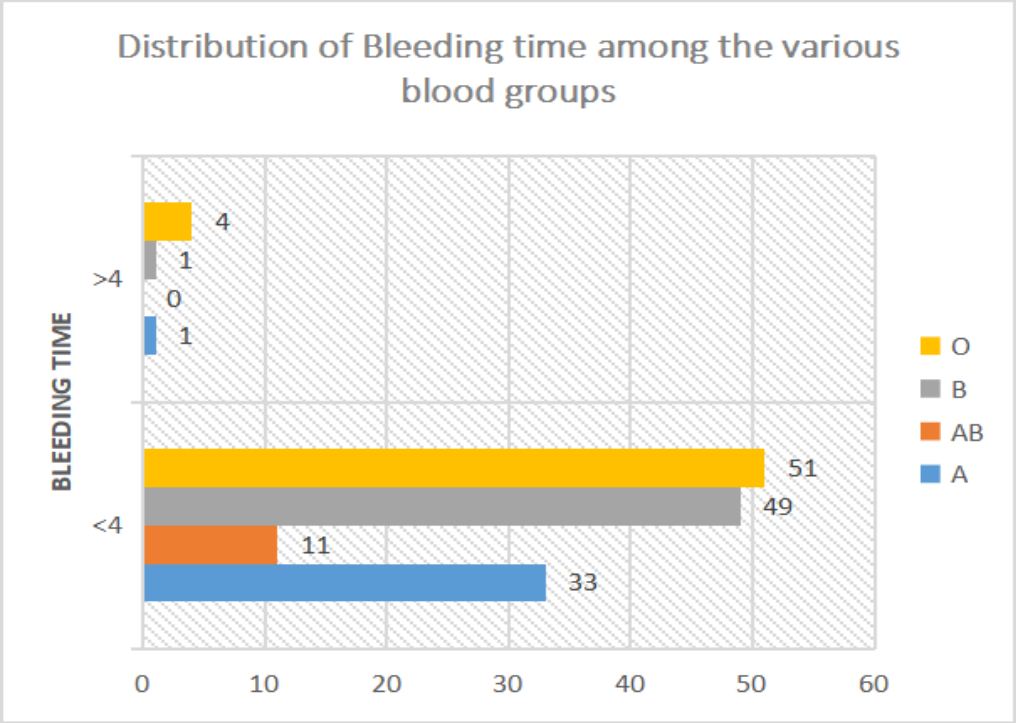
	A	AB	B	O	TOTAL
F	20	7	24	29	**80**
	25.00%	8.75%	30.00%	36.25%	**100.00%**
	58.82%	63.64%	48.00%	52.73%	**53.33%**
M	14	4	26	26	**70**
	20.00%	5.71%	37.14%	37.14%	**100.00%**
	41.18%	36.36%	52.00%	47.27%	**46.67%**
TOTAL	**34**	**11**	**50**	**55**	**150**
	22.67%	**7.33%**	**33.33%**	**36.67%**	**100.00%**
	100.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**



3.Distribution of Bleeding time among the various blood groups:

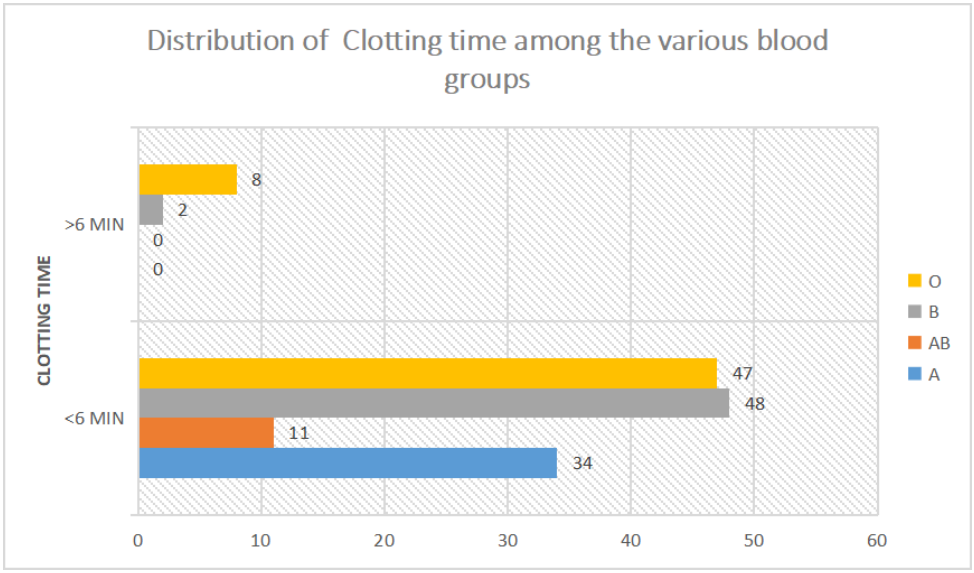
	A	AB	B	O	TOTAL

<4	33	11	49	51	**144**
	22.92%	7.64%	34.03%	35.42%	**100.00%**
	97.06%	100.00%	98.00%	92.73%	**96.00%**
>4	1	0	1	4	**6**
	16.67%	0.00%	16.67%	66.67%	**100.00%**
	2.94%	0.00%	2.00%	7.27%	**4.00%**
TOTAL	**34**	**11**	**50**	**55**	**150**
	22.67%	**7.33%**	**33.33%**	**36.67%**	**100.00%**
	100.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**



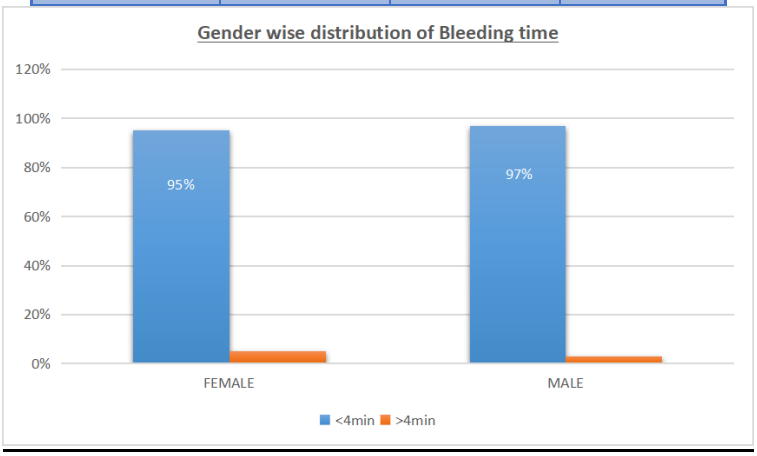
4.Distribution of Clotting time among the various blood groups:

	A	AB	B	O	TOTAL
<6	34	11	48	47	**140**
	24.29%	7.86%	34.29%	33.57%	**100.00%**
	100.00%	100.00%	96.00%	85.45%	**93.33%**
>6	0	0	2	8	**10**
	0.00%	0.00%	20.00%	80.00%	**100.00%**
	0.00%	0.00%	4.00%	14.55%	**6.67%**
TOTAL	**34**	**11**	**50**	**55**	**150**
	22.67%	**7.33%**	**33.33%**	**36.67%**	**100.00%**
	100.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**



5.Gender wise distribution of Bleeding time:

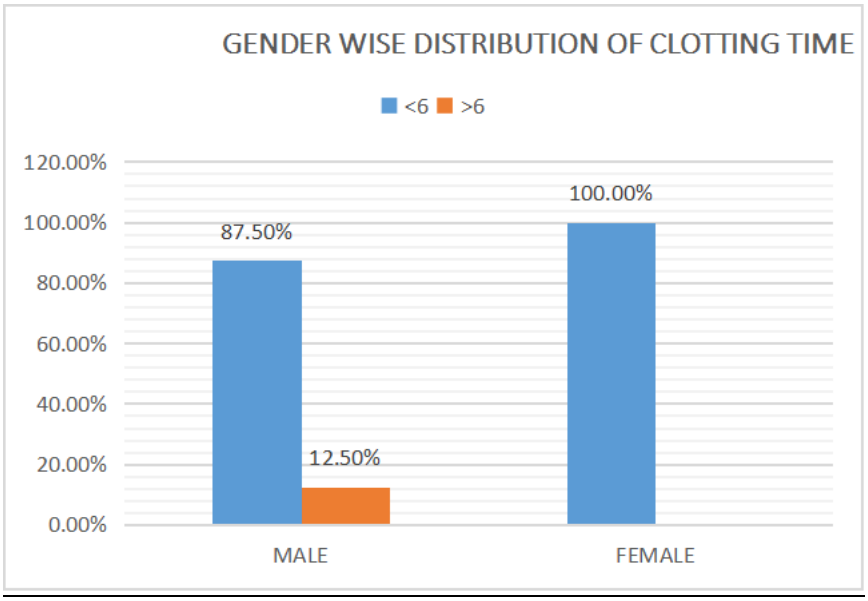
	BT ANALYSIS		
SEX	**<4**	**>4**	
F	76	4	80
Row %	95.00%	5.00%	100.00%
Col %	52.78%	66.67%	53.33%
M	68	2	70
Row %	97.14%	2.86%	100.00%
Col %	47.22%	33.33%	46.67%
Total	144	6	150
Row %	96.00%	4.00%	100.00%
Col %	100.00%	100.00%	100.00%



6. Gender wise distribution of Clotting time:

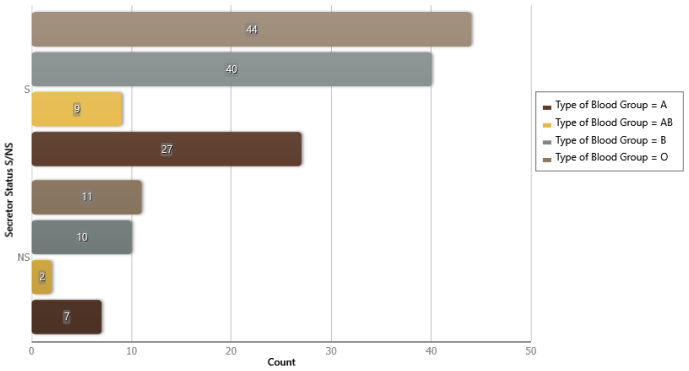
	CT ANALYSIS		
SEX	**<6**	**>6**	
F	70	10	80

Row %	87.50%	12.50%	100.00%
Col %	50.00%	100.00%	53.33%
M	70	0	70
Row %	100.00%	0.00%	100.00%
Col %	50.00%	0.00%	46.67%
Total	140	10	150
Row %	93.33%	6.67%	100.00%
Col %	100.00%	100.00%	100.00%



7. <u>Distribution of secretors and non secretors among students in various ABO blood groups among students:</u>

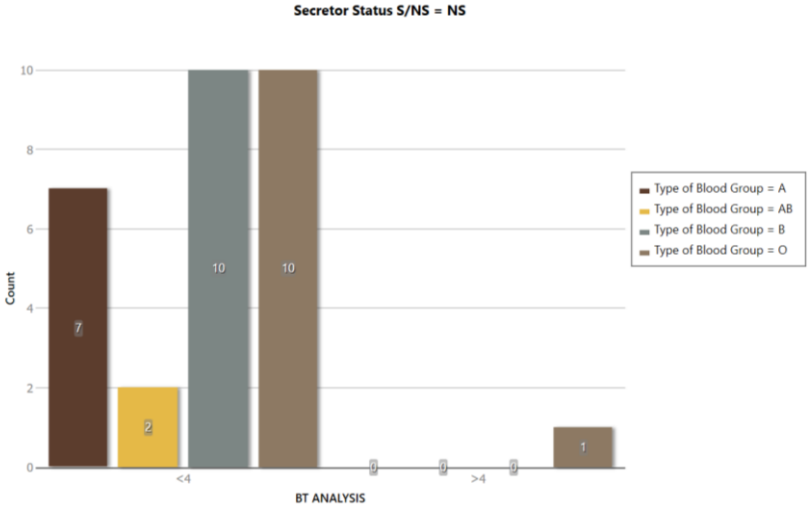
	A	**AB**	**B**	**O**	**TOTAL**
NS	7	2	10	11	**30**
	23.33%	6.67%	33.33%	36.67%	**100.00%**
	20.59%	18.18%	20.00%	20.00%	**20.00%**
S	27	9	40	44	**120**
	22.50%	7.50%	33.33%	36.67%	**100.00%**
	79.41%	81.82%	80.00%	80.00%	**80.00%**
TOTAL	**34**	**11**	**50**	**55**	**150**
	22.67%	**7.33%**	**33.33%**	**36.67%**	**100.00%**



8. <u>Distribution of BT among Secretors and NonSecretors in various ABO blood groups.</u>

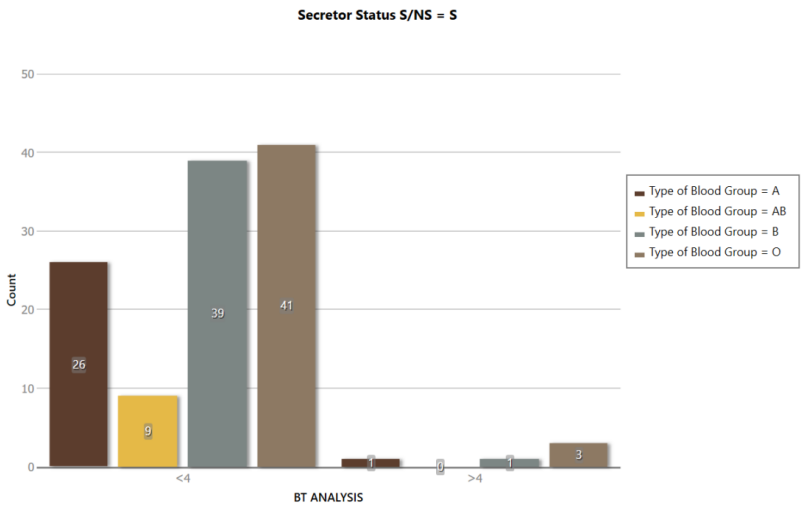
<u>Secretor Status S/NS = NS:</u>

	A	AB	B	O	TOTAL
<4	7	2	10	10	**29**
	24.14%	6.90%	34.48%	34.48%	**100.00%**
	100.00%	100.00%	100.00%	90.91%	**96.67%**
>4	0	0	0	1	**1**
	0.00%	0.00%	0.00%	100.00%	**100.00%**
	0.00%	0.00%	0.00%	9.09%	**3.33%**
TOTAL	**7**	**2**	**10**	**11**	**30**
	23.33%	**6.67%**	**33.33%**	**36.67%**	**100.00%**



<u>Secretor Status S/NS = S:</u>

	A	AB	B	O	TOTAL
<4	26	9	39	41	**115**
	22.61%	7.83%	33.91%	35.65%	**100.00%**
	96.30%	100.00%	97.50%	93.18%	**95.83%**
>4	1	0	1	3	**5**
	20.00%	0.00%	20.00%	60.00%	**100.00%**
	3.70%	0.00%	2.50%	6.82%	**4.17%**
TOTAL	**27**	**9**	**40**	**44**	**120**
	22.50%	**7.50%**	**33.33%**	**36.67%**	**100.00%**



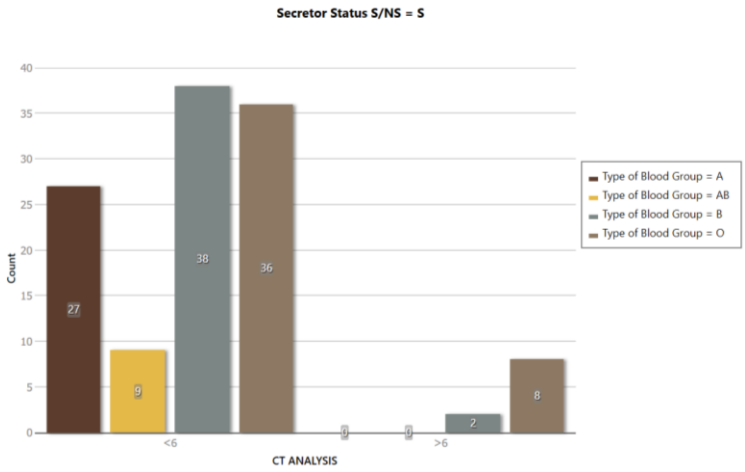
9. <u>Distribution of CT among Secretors and NonSecretors in various ABO blood groups.</u>

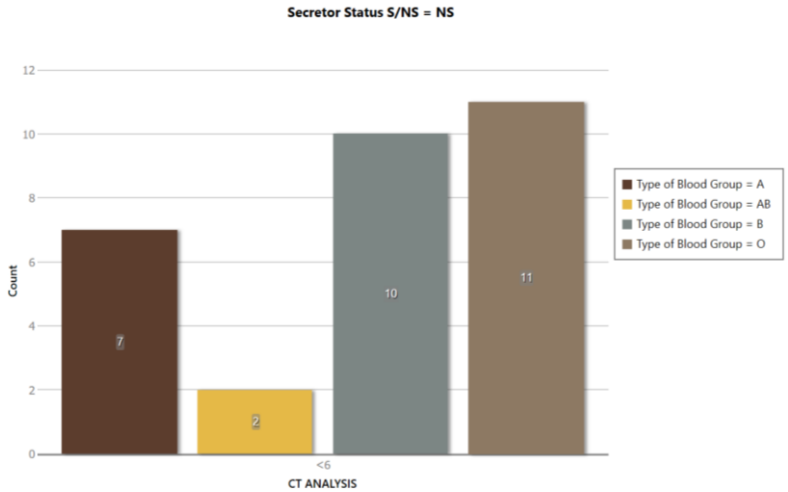
<u>Secretor Status S/NS = NS:</u>

	A	AB	B	O	TOTAL
<6	7	2	10	11	**30**
	23.33%	6.67%	33.33%	36.67%	**100.00%**
	100.00%	100.00%	100.00%	100.00%	**100.00%**
>6	0	0	0	0	**0**
	NaN	NaN	NaN	NaN	**NaN**
	0.00%	0.00%	0.00%	0.00%	**0.00%**
TOTAL	**7**	**2**	**10**	**11**	**30**
	23.33%	**6.67%**	**33.33%**	**36.67%**	**100.00%**

<u>Secretor Status S/NS = S:</u>

	A	AB	B	O	TOTAL
<6	27	9	38	36	**110**
	24.55%	8.18%	34.55%	32.73%	**100.00%**
	100.00%	100.00%	95.00%	81.82%	**91.67%**
>6	0	0	2	8	**10**
	0.00%	0.00%	20.00%	80.00%	**100.00%**
	0.00%	0.00%	5.00%	18.18%	**8.33%**
TOTAL	**27**	**9**	**40**	**44**	**120**
	22.50%	**7.50%**	**33.33%**	**36.67%**	**100.00%**





DISCUSSION:

Many studies have been done so far to correlate the association between the blood groups, bleeding time and clotting time. Very few studies have been done regarding the association of the secretor status and bleeding time and clotting times. According to the review article written by Massino Franchini et al, when compared to the type "O" blood group, the non "O" blood group individuals can have an increased risk of thrombosis due to the higher levels of vWF[25]. He also states that, the ABO group can affect the vWF

catabolism, means that the plasma vWF levels may depend upon the blood group type of the individual. This concept was accepted by other studies done by Jenkins PO et al,[26] who stated that vWF is 25% more in non O group individuals compared to O group individuals. This means that the clotting and bleeding time will be elevated among the "O" group individuals compared to the other groups. But as per the study done by Mahapatra et al[27] clotting time was prolonged in the group B individuals and bleeding time was prolonged in the AB group compared to other groups.

In our study the BT was prolonged in "O" group(66.67%) when compared to other groups. But this is not significant with Fishers exact 0.6416. CT was also prolonged in "O" groups(80%) compared to other groups. It is significant with Fisher exact 0.0431. BT(66.67%) and CT(100%) of females are prolonged when compared to BT(30.33%) and CT(100%) males and this is not significant (BT>4 min,CT >6 min). But in the study done by Mahapatra et al, there was no difference in bleeding time and clotting time among males and females.

Our study goes along with the study done by Roy B et al,[28] in which there is prolonged bleeding time and clotting time among females compared to males. This fact that the females individuals having comparatively increased BT and CT can be due to presence of estrogens, which as per Ercan M et al[29], decrease the level of fibrinogen in the plasma and increase in clotting time.

Among the ABO secretor status, in the present study, the secretors contrirbute for 80% and non secretors contribute 20%. Among ABO blood group types, "O" type blood group constitute (36.67%) maximum of the secretors. Among non-secretors also, type "O" blood group (36.67%) is maximum when compared to other groups in my study.

Secretor status and BT Analysis:

Among the secretors, type "O" blood groups have their bleeding time>4min to 60%. Type "O" blood group individuals have low VWF and have prolonged bleeding times, and are at high risk for epistaxis and in my study this is not significant with fisher's exact (0.8955). Secretors have longer BT and are at risk for bleeding tendencies. Among the non-secretors type "O", "B" blood groups have their BT <4min to 10%, 34.4% each and non-secrertors have high vWF and have more tendency for thrombotic tendencies.

Secretor status and CT Analysis:

Among secretors, type "B" have (34.55%) shorter CT<6 min. Among non secretors type "O" blood group (36.67%) have their CT<6 min. Non secretors having high VIII, VWF are at risk for future atherothrombotic and heart disease. The fisher exact is 1.000 and is not significant.

CONCLUSION AND RECOMMENDATIONS:

In this study, "O" group (36.67%) was the most common, while blood group AB was the least common(7.33%). Among the ABO secretor staus 80% are secretors and 20% are non-secretors. Gender wise BT and CT were higher in females than males.BT>4 min is maximum in type "O" secretors and have thinnest blood and are at risk for bleeding tendencies like epistaxis. CT<6min is more in B group secretors and are at high risk of thrombotic diseases like coronary artery disease and atherothrombotic diseases. Hence lifestyle modifications have to done to reduce the risk .

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