



## Correlation of Bleeding Time & Clotting Time with ABO bloodgroups

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### ABSTRACT

**Introduction:** Haemostasis is spontaneous arrest or stoppage of bleeding from damaged/ injured blood vessels. Evaluation of haemostasis is an essential factor before any surgery. The prerequisites are Bleeding Time (BT), & Clotting Time (CT) and these are also important in conditions like epistaxis, hemoptysis, GI bleeding, variceal bleeding. On the other side these are important for thrombotic risk and risk of cardiovascular disease.

**Objectives:** The objective of this study is to correlate BT,CT with Blood groups.

**Materials & Methods:** This is a cross sectional study conducted in department of Physiology, Osmania Medical College on 206 students of 1<sup>st</sup> year MBBS of both genders. Blood grouping was determined with standard antiserum. BT, CT with Duke method and capillary tube method respectively.

**Results:** In this study O bloodgroup was more prevalent(40.1%).Chi- square test applied, p-value is calculated, taking p-value<0.05 is significant. The correlation between bloodgroups and BT (p-value 0.05), bloodgroups and CT (p-value 0.03) were found significant. The values are prolonged in bloodgroup 'O' compared to other bloodgroups.

**Conclusion:** In present study blood group O was more common followed by B, A, AB. BT, CT prolonged in O blood group. BT, CT prolonged in females compared to males.

**Discussion:** BT, CT increased in females due to low hematocrit, & hormone oestrogen respectively. BT,CT increased in bloodgroup 'O' due to low plasma levels of vWF.

**Keywords:** Blood groups, Bleeding time, Clotting time.

- temporary haemostatic plug formation,
- definitive haemostatic plug formation.

### PLATELETS -

• Platelets are crucial for all the above processes by attaching to damaged collagen fibers and endothelial cells of vascular endothelium, releasing 5-HT to cause vasoconstriction, changing their morphology and releasing ADP, TXA2 to activate nearby platelets to adhere to them producing platelet plug. These also help in clot formation by helping in formation of intrinsic prothrombinactivator.

### BLEEDING TIME (BT)

• Bleeding time – The time lapse between the skin prick and the arrest of bleeding is called bleeding time.

### CLOTTING TIME (CT)

• Clotting time – The time taken by the fresh fluid blood to get coagulated (fibrin threads formation occurs).

- BT, CT are required to evaluate haemostasis before undergoing any surgical procedure.
- Their elevated levels are important in conditions like epistaxis, hemoptysis, &GI bleeding.
- Whereas decreased levels of the above factors are important in development of thrombosis & cardiovascular disease.
- Many studies have done to correlate Blood group with BT & CT.
- So that by knowing one's blood group we can expect some bleeding diathesis to some extent.

## I. INTRODUCTION

### HAEMOSTASIS-

- Haemostasis refers to spontaneous arrest or prevention of bleeding from injured vessels by the physiological processes –
- vasoconstriction,

### AIM-

TO STUDY CORRELATION OF BLEEDING TIME AND CLOTTING WITH ABO BLOOD GROUPS

- OBJECTIVES-



- To assess the distribution of blood groups
- and to study its correlation with bleeding time and clotting time.
- **METHODOLOGY**
- **STUDY DESIGN**- cross sectional study
- **STUDY POPULATION**- 1<sup>ST</sup> year MBBS students of Osmania medical college of age 18-30yrs
- **SAMPLE SIZE**- 206
- **INCLUSION CRITERIA**- students who have given consent
- **EXCLUSION CRITERIA**- bleeding diathesis, any acute illness

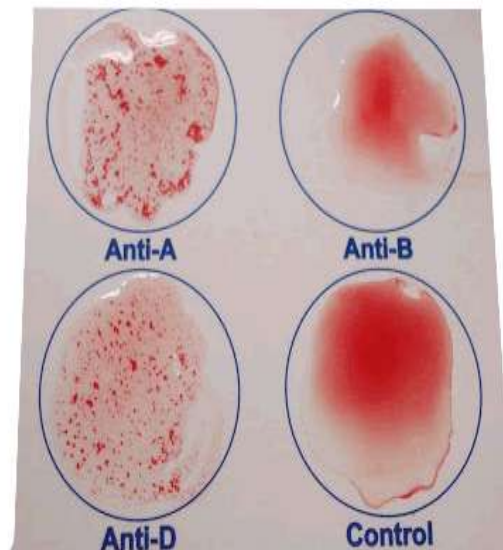
#### Materials and Methods –

This is a cross sectional study done in physiology department of Osmania Medical College, on 206 1<sup>st</sup> year MBBS students on both genders after taking consent.

- 1) Blood grouping – the ABO blood group system is the most important of all blood group systems.
- Procedure- under aseptic conditions a blood prick is given to finger, allowed to form a large drop. This was placed on the 3 slides, then mixed with a drop of antisera ‘anti-A’, ‘anti-B’, ‘anti-D’, slides are labelled about which antisera is mixed.
- The blood and antisera are mixed by gentle shaking the slides or mixed with separate sticks.
- Waited for 5 – 10 minutes, then checked for agglutination.
- These are identified as A, B, AB, O Blood groups and also Rh+ve, Rh-ve based on agglutination.
- On each slide a drop of blood is added to isotonic saline and marked it as control.

#### HOW TO READ YOUR RESULTS

| BLOOD TYPE  | ANTI-A       | ANTI-B       | ANTI-D       | CONTROL      |
|-------------|--------------|--------------|--------------|--------------|
| O-POSITIVE  | Red          | Red          | Agglutinated | Red          |
| O-NEGATIVE  | Red          | Red          | Red          | Red          |
| A-POSITIVE  | Agglutinated | Red          | Agglutinated | Red          |
| A-NEGATIVE  | Agglutinated | Red          | Red          | Red          |
| B-POSITIVE  | Red          | Agglutinated | Agglutinated | Red          |
| B-NEGATIVE  | Red          | Agglutinated | Red          | Red          |
| AB-POSITIVE | Agglutinated | Agglutinated | Agglutinated | Red          |
| AB-NEGATIVE | Agglutinated | Agglutinated | Red          | Red          |
| INVALID     | Agglutinated | Agglutinated | Agglutinated | Agglutinated |

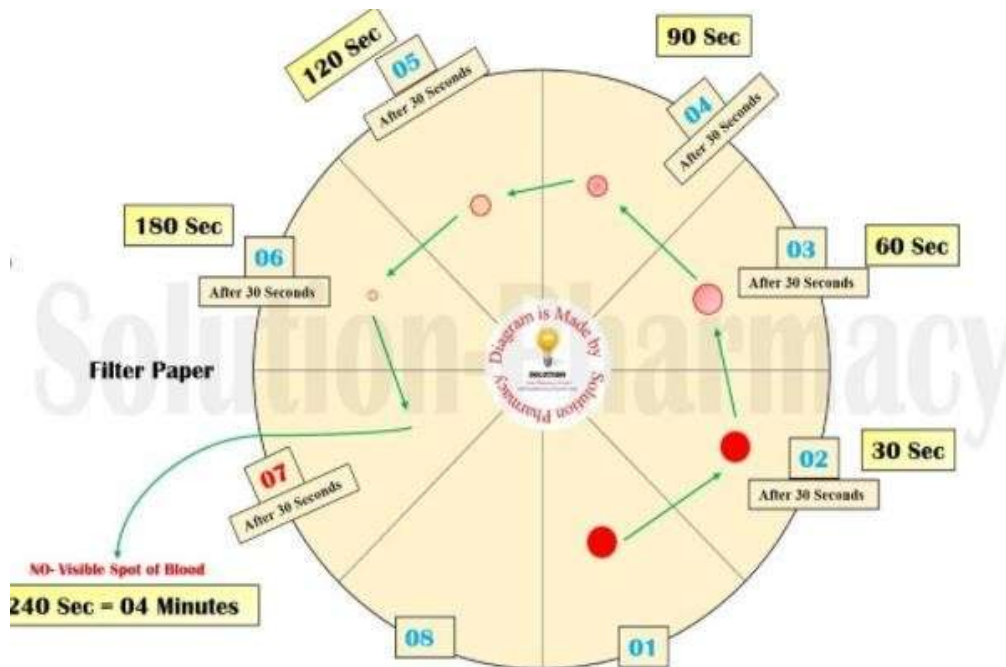


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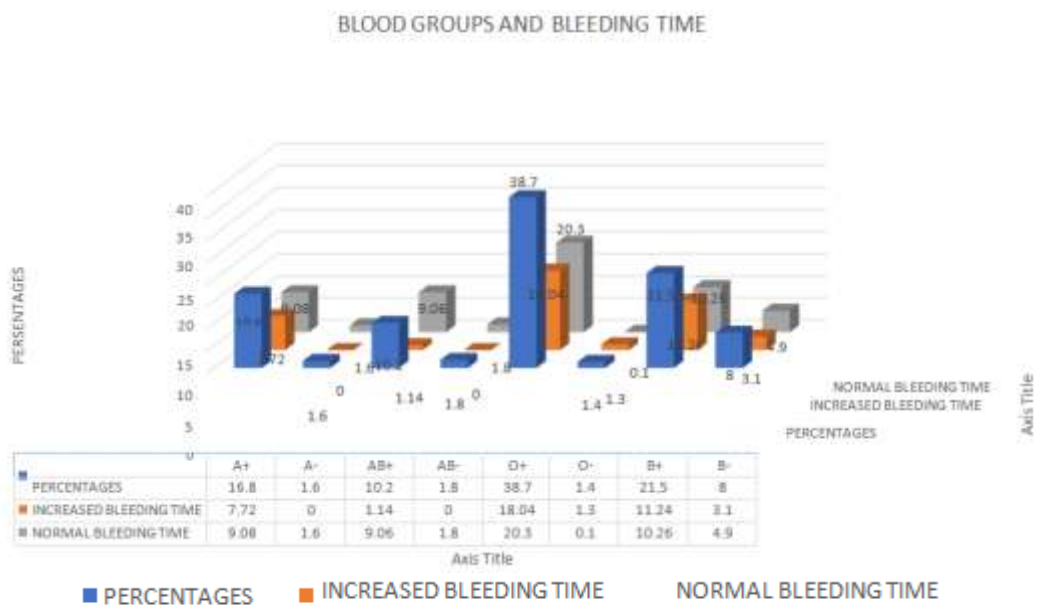
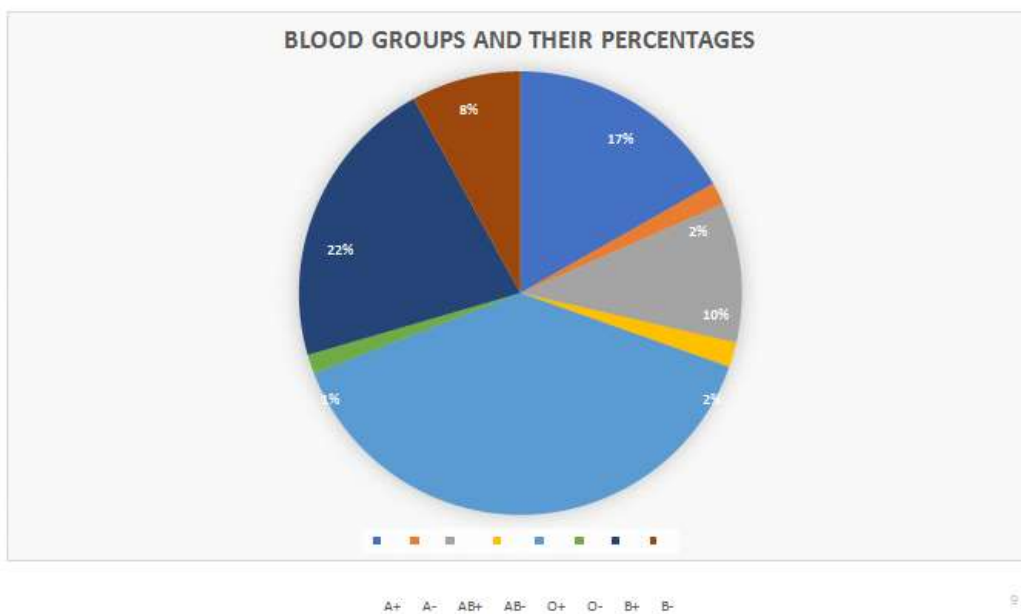
### BLEEDING TIME

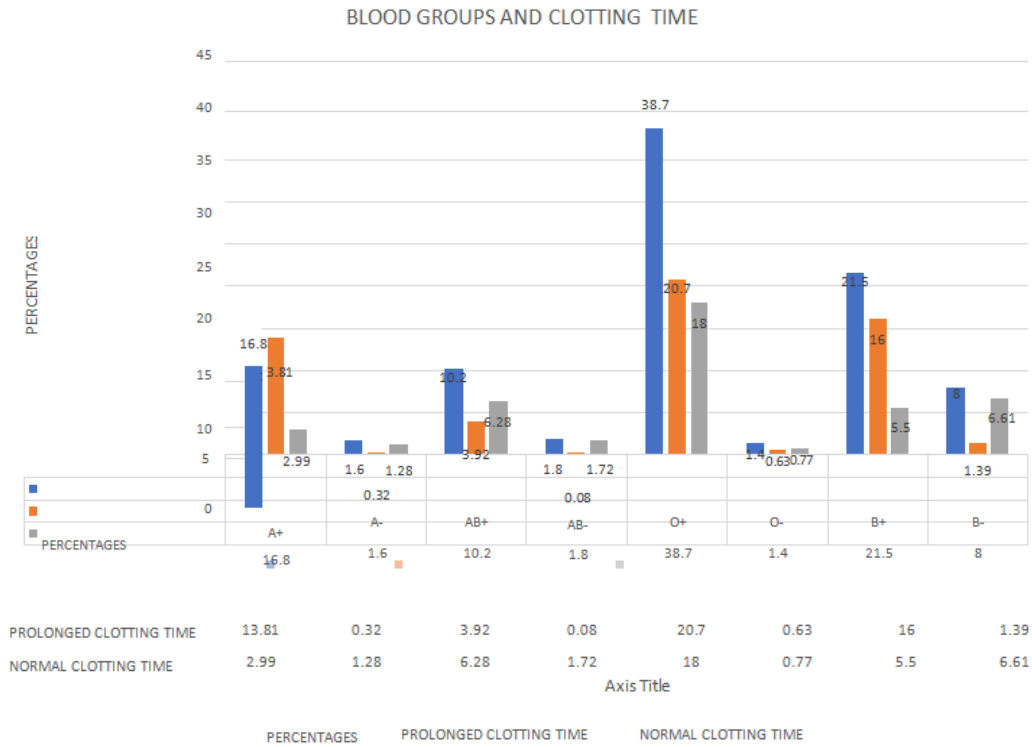
- **DUKE METHOD-** A deep skin puncture is given and length of time required to stop bleeding is recorded. It determines function of platelets and integrity of capillaries.
- Under aseptic precautions, a prick is given on tip of finger, stopwatch started.
- Blotting done for every 30 seconds on blotting paper.
- Stopwatch is stopped after bleeding stops, the number of drops on blotting paper counted and multiplied by 30.
- Normal range is 1 to 5 min.



### CLOTTING TIME

- **CAPILLARY TUBE METHOD-** this method is routinely used in clinical laboratories.
- Under aseptic precautions a skin prick is given, the blood is taken into a capillary tube. The length of the time taken for the blood to clot is reported as clotting time.
- Capillary tube was held between the palms to maintain it at body temperature.
- After 2 minutes, 1-2 cm capillary tube broken at from one end, and then for every 30 seconds and looked for fibrin thread formation. A thin string of fibrin formed between the broken ends.
- Stopwatch stopped and time is noted.
- Normal range is 2-8 minutes





**CHI-SQUARE TEST AND p-value calculation**

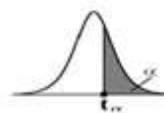
$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

$\chi^2$  = the test statistic       $\sum$  = the sum of

O = Observed frequencies      E = Expected frequencies



Percentage Points of the *t* Distribution;  $t_{v, \alpha}$   
 $P(T > t_{v, \alpha}) = \alpha$



| v  | $\alpha$ |       |       |       |       |       |        |        |        |        |        |        |         |         |
|----|----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|---------|---------|
|    | 0.40     | 0.30  | 0.20  | 0.15  | 0.10  | 0.05  | 0.025  | 0.02   | 0.015  | 0.01   | 0.0075 | 0.005  | 0.0025  | 0.0005  |
| 1  | 0.325    | 0.727 | 1.376 | 1.963 | 3.078 | 6.314 | 12.706 | 15.895 | 21.205 | 31.821 | 42.434 | 63.657 | 127.322 | 636.590 |
| 2  | 0.289    | 0.617 | 1.061 | 1.386 | 1.886 | 2.920 | 4.303  | 4.849  | 5.643  | 6.965  | 8.073  | 9.925  | 14.089  | 31.598  |
| 3  | 0.277    | 0.584 | 0.978 | 1.250 | 1.638 | 2.353 | 3.182  | 3.482  | 3.896  | 4.541  | 5.047  | 5.841  | 7.453   | 12.924  |
| 4  | 0.271    | 0.569 | 0.941 | 1.190 | 1.533 | 2.132 | 2.776  | 2.999  | 3.298  | 3.747  | 4.088  | 4.604  | 5.598   | 8.610   |
| 5  | 0.267    | 0.559 | 0.920 | 1.156 | 1.476 | 2.015 | 2.571  | 2.757  | 3.003  | 3.365  | 3.634  | 4.032  | 4.773   | 6.869   |
| 6  | 0.265    | 0.553 | 0.906 | 1.134 | 1.440 | 1.943 | 2.447  | 2.612  | 2.829  | 3.143  | 3.372  | 3.707  | 4.317   | 5.959   |
| 7  | 0.263    | 0.549 | 0.896 | 1.119 | 1.415 | 1.895 | 2.365  | 2.517  | 2.715  | 2.998  | 3.203  | 3.499  | 4.029   | 5.408   |
| 8  | 0.262    | 0.546 | 0.889 | 1.108 | 1.397 | 1.860 | 2.306  | 2.449  | 2.634  | 2.896  | 3.085  | 3.355  | 3.833   | 5.041   |
| 9  | 0.261    | 0.543 | 0.883 | 1.100 | 1.383 | 1.833 | 2.262  | 2.398  | 2.574  | 2.821  | 2.998  | 3.250  | 3.690   | 4.781   |
| 10 | 0.260    | 0.542 | 0.879 | 1.093 | 1.372 | 1.812 | 2.228  | 2.359  | 2.527  | 2.764  | 2.932  | 3.169  | 3.581   | 4.587   |
| 11 | 0.260    | 0.540 | 0.876 | 1.088 | 1.363 | 1.796 | 2.201  | 2.328  | 2.491  | 2.718  | 2.879  | 3.106  | 3.497   | 4.437   |
| 12 | 0.259    | 0.539 | 0.873 | 1.083 | 1.356 | 1.782 | 2.179  | 2.303  | 2.461  | 2.681  | 2.836  | 3.055  | 3.428   | 4.318   |
| 13 | 0.259    | 0.538 | 0.870 | 1.079 | 1.350 | 1.771 | 2.160  | 2.282  | 2.436  | 2.650  | 2.801  | 3.012  | 3.372   | 4.221   |
| 14 | 0.258    | 0.537 | 0.868 | 1.076 | 1.345 | 1.761 | 2.145  | 2.264  | 2.415  | 2.624  | 2.771  | 2.977  | 3.326   | 4.140   |

### CHI-SQUARE TEST & p-value Clotting time

| GENDER | CHI SQUARE | VALUE | df | P- value     |
|--------|------------|-------|----|--------------|
| FEMALE | CHI        | 2.416 | 7  | <u>0.025</u> |
|        | LR         | 5.270 | 7  | 0.416        |
|        | N          | 132   |    |              |
| MALE   | CHI        | 2.572 | 6  | <u>0.034</u> |
|        | LR         | 7.876 | 6  | 0.392        |
|        | N          | 74    |    |              |
| TOTAL  | CHI        | 2.569 | 7  | <u>0.03</u>  |
|        | LR         | 7.635 | 7  | 0.408        |
|        | N          | 206   |    |              |



## Bleeding time

| GENDER | CHI SQUARE | VALUE | df | P -value     |
|--------|------------|-------|----|--------------|
| FEMALE | CHI        | 2.645 | 7  | <u>0.038</u> |
|        | LR         | 7.954 | 7  | 0.356        |
|        | N          | 132   |    |              |
| MALE   | CHI        | 2.089 | 6  | <u>0.056</u> |
|        | LR         | 6.856 | 6  | 0.361        |
|        | N          | 74    |    |              |
| TOTAL  | CHI        | 1.943 | 7  | <u>0.05</u>  |
|        | LR         | 4.894 | 7  | 0.541        |
|        | N          | 206   |    |              |

### II. CONCLUSION

- In the present study blood group 'O' was more common followed by B, A, AB.
- CLOTTING TIME prolonged in 'O' blood group, statistically significant.
- And CT is also prolonged in B,(after'O') but statistically not significant.
- BLEEDING TIME is prolonged in 'O' blood group persons which is statistically significant.
- It is also prolonged in B, but it is not significant statistically.
- When compared to males, females has more bleeding time & clotting time.

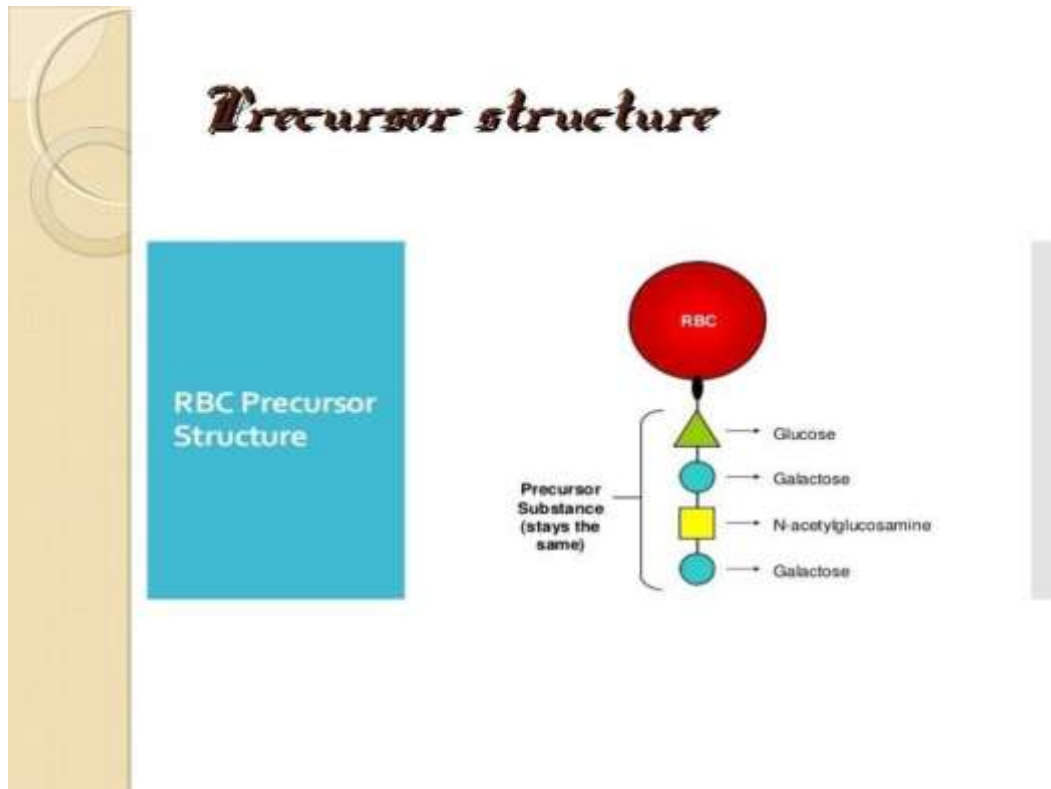
### III. DISCUSSION

- The ABO Blood group is the most prevalent important blood group system in human.
- The gene is located on chromosome 9, it is a single gene with 3 types of alleles (i, IA, IB).
- This gene encodes a glycosyltransferase.

- This is found not only in RBC, but also found on platelets, endothelium, and in many cell types.
- ABO blood group is a major determinant of plasma levels of vWF and factor 8.
- ABO exerts major quantitative & qualitative effects on vWF, also effects specific aspects of platelet function.
- vWF is one of the several components of the coagulation system.
- The gene for vWF is on chr.12, but ABO gene exerts major quantitative effects on plasma levels of this factor.
- It is essential to know how ABO gene is exerting its influence on vWF, and function of platelets.
- To explain this, the biochemical structure of antigens for A, B, AB, O blood groups should be studied.



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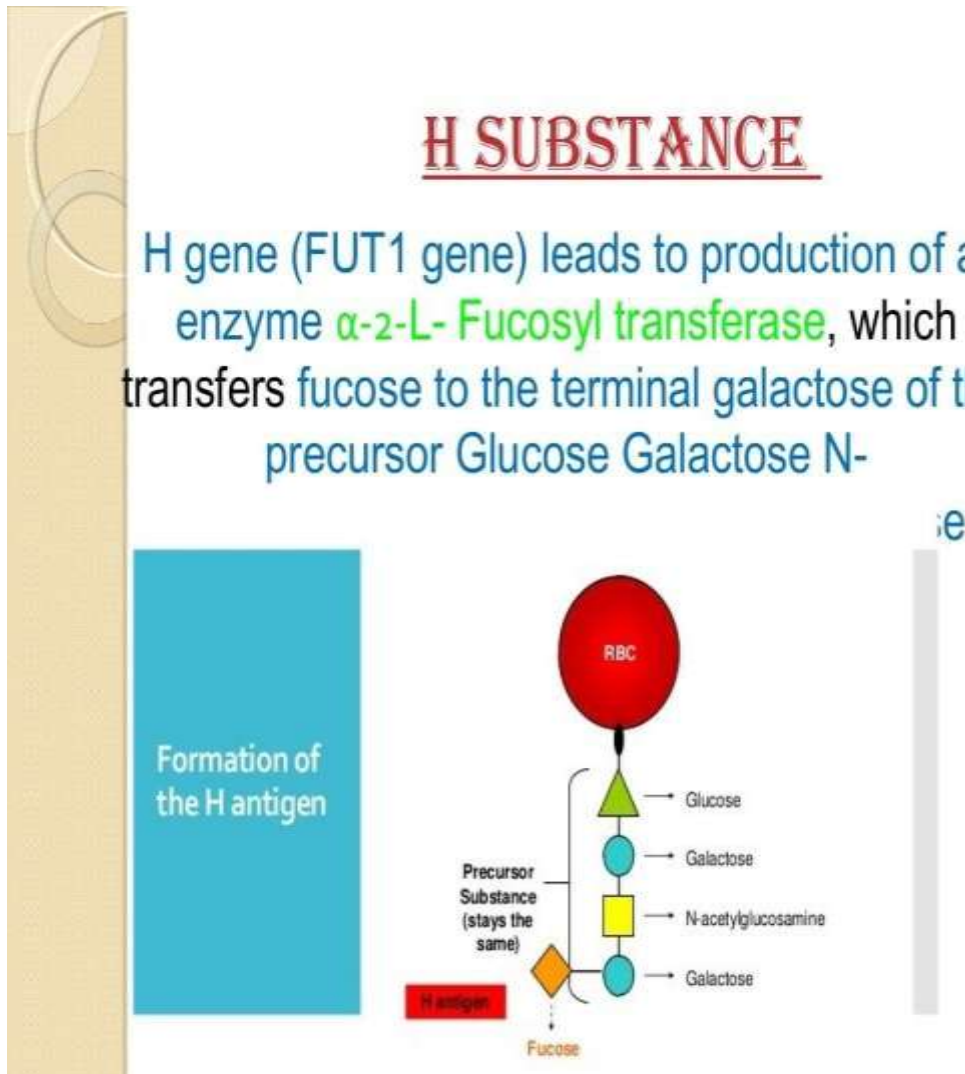






## H SUBSTANCE

H gene (FUT1 gene) leads to production of an enzyme  $\alpha$ -2-L- Fucosyl transferase, which transfers fucose to the terminal galactose of the precursor Glucose Galactose N-



- ABO antigens has complex carbohydrate molecules , ABO alleles encodes for Glycosyltransferases to a common precursor sidechain, the H determinant, converting it to A/B.
- 'O' blood group don't encodes a functional enzyme. So, it continues to express the basic, unmodified, H-Ag with a fucose moiety attached to precursor oligosaccharide chain.
- In 'O' blood group, aberrant expression of Glycosyltransferase results in altered vW F glycosylation, structural changes, these results in increased clearance and therefore lowlevels of plasma vWF.
- And in 'O' group individuals, because of increased activity of ADAMTS13, vW F breakdownfast.
- Normal value of vWF – 100IU/dl
- In 'O' it is – 75IU/dl
- Half life normal- around 16 hours
- In 'O' it is – around 4.2 hours
- At the site of vascular injury, vWF attaches with exposed collagen with it's A3 domain.
- Then it interacts with GPIb alpha on platelets causing them to adhere to wound sites.
- It also binds with factor VIII, it protects Factor VIII from degradation by thrombin.
- It also binds with Thrombin in coagulation pathway.

In 'O' blood group persons as they have low plasma level of vWF and increased clearance of it results in increase in intrinsic and common pathway duration resulting in increased clotting time duration.

- Due to decreased adhesion and activation of platelets on damaged vascular surface, leads to decreased release of vasoconstrictors like 5-HT, and decreases release of ADP, TXA2 leads



to delay in the platelet plug formation leading to increased bleeding time as seen in 'O' blood group persons.

- Bleeding time is more in females probably due to low haematocrit and low skin temperature.
- Clotting time is also more in females due to the presence of hormone estrogen which leads to decreased fibrinogen levels.

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