ABO BLOOD GROUPING FROM HARD AND SOFT TISSUES OF TEETH BY MODIFIED ABSORPTION-ELUTION TECHNIQUE

A Dissertation submitted in

partial fulfillment of the requirements

for the degree of

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BRANCH – IX

ORAL MEDICINE AND RADIOLOGY

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

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DECLARATION

TITLE OF DISSERTATION	ABO blood grouping from hard and soft tissues of the teeth by modified absorption-elution technique
PLACE OF STUDY	K.S.R. Institute of Dental Science and Research
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NAME OF THE GUIDE	Dr.(Capt) S.Elangovan
HEAD OF THE DEPARTMENT	Dr.(Capt) S.Elangovan

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DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

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This is to certify that **Dr. A.NILOPHAR,** Post Graduate student (2013-2016) in the Department of Oral Medicine and Radiology , K.S.R. Institute of Dental Science and Research, has done this dissertation titled "**ABO BLOOD GROUPING FROM HARD AND SOFT TISSUES OF TEETH BY MODIFIED ABSORPTION-ELUTION TECHNIQUE**" under our guidance and supervision in partial fulfillment of the regulations laid down by the **Tamilnadu Dr.M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,(**Branch – IX) **Oral Medicine and Radiology** degree examination.

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PRINCIPAL

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INTRODUCTION

INTRODUCTION

Forensic identification by its nature is a multidisciplinary approach relying on positive identification methodology as well as presumptive or exclusionary methodologies. This is a branch, which deals with identification and has many maxims, the best known of which, is that every contact leaves its trace. Typically this effect involves the cooperation and co-ordination of law enforcement officials, forensic pathologists, forensic odontologists, serologists and criminologists[1]

Federation Dentaire International FDI has defined forensic odontology as that branch of dentistry which in the interest of justice deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings. Dr.Oscar Amoedo is regarded as the father of forensic odontology. [2]

Blood grouping has been one of the corner stones of identification of biological material. The term blood group is applied to the presence of inherited antigens on the red cell surface by specific antibodies.[3]

In 1900 Austrian- American physician Dr. Karl Land Steiner first described ABO blood grouping .A person's ABO type depends upon the presence of two genes - the A and B genes and these genes are encoded on chromosome 9 (in band 9q34.1). They determine part of the configuration of the red blood cell surface. A person can be A, B, AB, or O. If a person has two A genes, their red blood cells are type A. If a person has two B genes, their red cells are type B. If the person has one A and one B gene, their red cells are type AB. If the person has neither the A nor B gene, they are type O.[4]

The use of blood group antigens in forensic science is based upon the fact that once if a blood group is established in an individual it remains unchanged

throughout his/her life. The presence of these antigens in dental tissues helps in identifying the highly decomposed bodies in which the bones and teeth are significantly remaining tissues. [5]

Pulp tissue is enclosed within the dental hard tissues, where post-mortem changes are seen very late. Since tooth pulp is highly vascular, blood group antigens are most certainly bound to be present. In dentin, these substances are located in the dentinal tubules. The possible distribution of ABO substances from the pulp cavity wall to the dentin edge and to the enamel gradually decreases because of fewer possibilities of diffusion of antigens from both blood and saliva.[6]

The presence of ABO blood grouping antigens in pulp and dentin make it possible to determine the blood group typing and thereby assist in identifying even a highly decomposed body. Recent tooth specimens are expected to provide good results. However, certain effect on the tissues like autolysis, dehydration, loss of pulp antigens or high number of errors due to foreign antigen borne by bacteria in carious teeth may lead to variation in the study.[7]

Absorption–elution (AE) technique was devised by Siracusa[8] in 1923 and has been modified by Kind[9]. Various modifications have taken place since then to improve the sensitivity. This technique has been used acceptably to determine blood group from dried stains, tissues, secretions, and teeth in various forensic laboratories. This method is more sensitive, highly specific, and least interfered with the nature of the substrata. It has been found that the material once used is available for reuse with practically no loss in its antigenic property by addition of certain substances.[9]

Teeth are considered as invaluable source of personal identification in mass disasters, where the recognition of an individual could prove to be difficult. Teeth are resistant to environmental assaults, such as, mutilation incineration, immersion,

trauma and decomposition. Therefore teeth represent an excellent source for identification. Blood grouping from various methods from the teeth could be a source of personal identification [10.]

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To determine the blood group from pulp and dentin, from the extracted teeth by modified absorption –elution technique and to correlate the results with the reference sample which was obtained by slide agglutination method
2. To evaluate the reliability of teeth stored for a period of 4-6 months as a source of blood group substances.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Ivor R, Kramer H (1957)[11] conducted a study using dentin powder, which was prepared from newly extracted teeth after decalcification technique. They used a simple modification of the mixed agglutination technique for demonstration of A and B antigens. No dentine powder-erythrocyte agglutination could be detected. They concluded that it was not possible to demonstrate these substances in human dentin.

Kind S.S (1962)[12] conducted experiments to detect the presence of antigens and antibodies from dried blood stains. Using blood stained fabrics agglutinins were detected and the failure for results was mainly due to absorption or deterioration of agglutinins. This was due to temperature changes and at that time favorable results were obtained if the blood stained articles were preserved by storing them with calcium chloride The detection of agglutinogens was done by two different process, the absorption inhibition process and absorption-elution technique. The later technique gave better results than the former one.

Outteridge R. A (1963)[13] conducted a study where a comparison was made between the absorption-elution and the absorption-inhibition methods of grouping dried bloodstains in the ABO system. The two methods are compared on the basis of sensitivity, reliability and ease of use in the laboratory. On all counts the elution method is the method of choice. It was much more sensitive than the inhibition method and also it was simple and flexible which can be easily used in the laboratories.

Vitullo L.R (1967)[14] stated that the use of enzyme treated cells in detecting the blood group antigens showed results with high degree of sensitivity. Though certain enzymes like trypsin and ficin showed some limitations, bromelin a

group of enzymes which was derived from pineapple stem showed 3+, 4+ agglutination when it was eluted with serum and erythrocytes. Out of 55 samples, 51 samples or 92.7% showed positive results when used with bromelin and this is almost 13% more when compared with absorption elution method.

Suyama H, Ohya I, Fukae T, Imai T (1976)[15] worked on investigation of a 42 year old man who died from hemorrhage resulting from stab wounds in his neck, chest and abdomen where he was transfused with 4000 ml of blood. According to laboratory findings both the victim and the assailant had the same blood group. For the purpose of distinguishing the victim's blood stains from the assailant's, the MN blood group and the isoenzymic phenotypes such as PGM, and 6-PGD were done and was proved to be M because the tests performed on blood stains at the scene of the crime corresponded with the tests carried out on his dental pulp, peripheral nerve and bone marrow by means of PGM $_1$ phenotyping. The victim's dental pulp, peripheral nerve and bone marrow were unaffected by the transfused blood.

J.B.Mukherjee, P.K.Chattopadhyay (1976)[16] analyzed the remains of a deceased person which were recovered three years after he was reported missing. The teeth were subjected to examination for the detection of blood group specific substances. The blood group of the subject was found to be AB. The individual peculiarity of disposition of teeth with remarkable similarity to the other members of the family played a leading role in establishing the identity of the person.

Korszun A.K, Causton B.E, Lincoln P.J (1978)[17] studied the thermostability of ABO blood group antigens in dental pulp using differential scanning calorimeter. 30 non carious teeth were taken from donors of ABO blood group. The heat diffusion study shows that dentin and enamel were poor insulators, and give inadequate thermal protection to the pulp when the external temperature rises

to 200 ^{0}C or more. Therefore in 200 ^{0}C only those teeth protected by the tongue and cheeks or by bone would be expected to exhibit ABO antigen activity. Out of three antigens tested, B antigen showed greatest resistance to heat.

Scott L.M, Corry J.E.L (1980)[18] conducted study in three saliva samples with false positive ABO grouping results and they were assayed for blood group active organisms, using a variety of selective media to determine the presence of salivary microflora of particular strains. Eight out of 40 in two samples and 4 out of 30 strains from the third sample showed blood group activity, which correlated well with the false positive specificities of the saliva samples. Hence the occasional false positive ABO grouping reactions of saliva samples are probably due to the presence of unusually high numbers of blood group active micro-organisms which causes disturbances in the ecological balance of the salivary microflora.

Arun Ganguli (1984)[19]presented a case report where the analysis of blood typing was used as a means of establishing identity. Dental hard tissue, pulp and alveolar bone were utilized. In conclusion the presented case showed that the pulpal tissues as well as alveolar bone contained sufficient blood cells to ascertain the blood groups of the victims.

James.L.Mudd (1986)[20]described a sensitive and reliable heamagglutination assay, using V bottom micro plates, for the detection of ABO blood group antibodies in blood stained material. When used in conjunction with absorption –elution procedure, the micro plate assay resulted in 300% increase in the number of conclusive grouping results when compared to the lattes crust test .The use of the micro plate reverse grouping assay permits 24 specimens to be assayed conveniently on a single plate and eliminates the tedious and time –consuming microscopic examination required for the lattes crust.

Kaur G, Sharma V.K (1988)[21] analyzed 72 sweat stain samples for determination of ABO blood grouping by absorption inhibition and absorption-elution technique. Also saliva was collected from the same individuals to reveal the presence of antigens in saliva and the secretor status was compared between the two variables. The study concluded that 95.84% were secretors in sweat stains using absorption-elution technique and 87.50 % were secretors in saliva.

Hooft P, Voorde H, Dijik P.V (1991)[22] in a study, isolated gram-negative aerobic oral bacterial flora from 100 consecutive corpses. After the identification and culturing of the isolated organisms, blood grouping was performed by the haemagglutination inhibition technique on the dried culture medium, dried culture smears, and dried ethanol extract of the bacteria. Forty-seven of the samples showed a gram-negative aerobic bacterial growth where 58 microorganisms of 14 different species were found. In Escherichia coli and Serratia marcescens positive blood grouping results were found and it was type B. So, the occasional mistyping of blood groups on saliva and oral soft tissues may be caused by the oral gram-negative aerobic flora, especially if the specimens are contaminated or putrefying.

Smeets B, Voorde H, Hooft P (1991)[6] conducted a study, where the ABO blood group was determined on the pulp, dentin and enamel of 35 teeth using adsorption-elution technique. Blood sample obtained from the extraction wound was used as the reference sample. Twenty teeth were examined within 6 weeks after extraction and fifteen teeth were examined 6–10 months after extraction. It was found, that the blood grouping on pulp gives fairly good results, whereas from enamel and dentin the results were limited. Similar results were found in both groups of teeth.

Sharma A.K, Chattopadhyay P.K (1993)[23] studied in dental tissues namely enamel, dentine, pulp and cementum from 53 permanent molars were

analyzed for the presence or absence of ABO blood group and other genetic markers like phosphoglucomutase (PGM,), glyoxalase (GLO-I), esterase D (EsD), erythrocyte acid phosphatase (EAP), adenylate kinase (AK), adenosine deaminase (ADA), 6-phosphogluconate dehydrogenase (6-PGD), glucose-6-phosphate dehydrogenase (G-6-PD) and carbonic anhydrase I1 (CA-11) isozymes simultaneously from teeth within a week of their collection. Dentine, pulp and cementum samples from155 teeth (including 14 deciduous teeth) were analyzed, two weeks after their collection, for ABO blood groups, and the different iso-enzymes were determined from the dental pulp only. Teeth stored at room temperature for a period of up to 90 weeks were also analyzed at different intervals to observe the persistence of the different genetic markers. No positive reaction for ABO substances were observed from enamel samples. The other dental tissues gave reactions for ABO substances even after 21 months storage at room temperature. Pulp tissue showed the most intense reaction. All dentine samples were also typed correctly, but the reactions were less intense when compared to that of pulp.

Nakayama Y, Aoki Y (1998)[24] designed a study to investigate localization of ABO antigens on the inner surface of human tooth hard tissues. Scanning electron microscope studies showed that the inner surface was exclusively covered with odontoblastic zone and no blood vessels remained in the samples. Blood group activities of the tooth fragments, which were detected with absorption elution test, were decreased when odontoblatic zone was scraped off. The results of these experiments indicate that the odontoblasts are one of the potent sources of blood group antigenicity for blood grouping of human teeth.

Kobayashi T, Yokota M, Mitani T, Akane A (1999)[25] used commercially available monoclonal antibodies for ABO blood grouping from

specimens like bloodstains, salivary stains, seminal stains, nails, hair and cerebral dura mater. The procedure was performed with an absorption-elution test. In certain substances the blood group was not determined correctly. When the antibody solvents were displaced with 5-20 % bovine serum albumin in saline, human serum of group AB donar or serum of sheep, chicken or bovine, titers of the reagents increased 2-8 times. The sensitivity and specificity of the tests were improved by the addition of these reagents.

Shetty M, Premalatha K (2010)[26] conducted a study in K S Hegde Medical Academy, Mangalore where 60 extracted teeth were collected from dead bodies brought to the Mortuary for medico legal autopsies. The age ranging from 14-60yrs. 31 males and 29 females were selected for the study. The extracted teeth were stored for a span of 6months at the room temperature without any preservative. The blood grouping was performed by absorption elution test using pulp and dentin which was later compared with the recorded blood group from the extracted socket. It was concluded that blood grouping on tooth pulp might be of great help in identification even after six months of death but the results of blood grouping on dentin would seem to be of limited value.

Ballal S, David M.P (2011)[10] conducted a study in 30 extracted teeth which was stored for 6 months. Blood group from dentin and pulp was made by absorption–Elution method. The pulp showed positive results for 27 out of 30 teeth and the sensitivity was 90%. Two teeth showed negative results, where as one tooth showed mistyping of blood grouping. Blood grouping from dentin was not correlating with control group. Hence, it was concluded that the dental pulp can be used to establish identity, where teeth happen to be the only remnant source available for

personal identification. Dentin may not be reliable source for blood group determination according to this study.

Aswath N, Selvamuthukumar S.C, Karthika B (2012)[27] conducted a study in 60 patients to determine the blood grouping and rhesus factor from pulp. The samples obtained from finger-pick method from those patients were considered as control and the samples obtained from the pulp were considered as case. The blood grouping was done by absorption-elution technique. Fifty seven teeth out of sixty showed positive results. Blood group elicited from slide‑agglutination method matched with that of the pulpal blood group elicited by absorption‑elution method. Thus, the high potential value of dental pulp tissue is highlighted in this study.

Karthika B, Elumalai M (2013)[4] reviewed various studies to identify the blood group from dentin and pulp, where they found that the teeth being the hardest tissue in the body, retain their characteristics even in the most adverse environmental conditions. While the other means of identification like facial and dermatoglyphic characteristics, tattoos, marks etc fail due to mutilation, decomposition and charring. The pulp tissue present inside the root canal which carries blood vessels and nerves is one of the most protected tissues of the oral tissues as it is surrounded on all sides by other dental hard tissues. Hence pulpal tissue from teeth is used for blood grouping and is considered as a hallmark for identification of biological material in forensic investigation.

Ramnarayan B.K, Manjunath M,Joshi A.A (2013)[3] conducted a study in 60 teeth where the samples were equally divided into two different groups. In the first group the results were obtained within a span of 6 weeks where as in the second group the results were obtained after 6 months. The grouping was done by modified absorption elution technique. Blood group obtained from the teeth was

compared with those obtained from the blood sample. Pulp showed a very large correlation in both fresh and long‑standing teeth though it decreased slightly in the latter. Hard tissue showed a large correlation in both the groups indicating that hard tissue is quite reliable to detect blood group and that there is no much difference in the reliability in both the groups. However, combining pulp and hard tissue, correlation is moderate.

Pai K.R, Tellis R et al (2014)[28] collected 50 exfoliated primary teeth and it was stored at varying pH (4, 7, and 10), sea water and buried in soil for 6 months and preserved at 4^0 C till DNA extraction was carried out.DNA was subjected to PCR amplification monoplex allele – specific PCR primers for ABO genotyping. Overall 66 % samples showed a DNA yield. Hundred percent results were obtained for samples studied at pH 4 and pH 7 whereas samples stored at pH 10 showed 10% result. Sea water and buried samples showed 80 % and 40 % results respectively.

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MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCE OF DATA

The present study was conducted in K.S.R Institute of Dental Science and Research, Tiruchengode where the extracted teeth were collected from patients who underwent dental extraction procedure.

INCLUSION CRITERIA

Hundred clinically sound human permanent teeth were collected randomly along with the respective blood samples from patients with age ranging 11-70 who reported for extraction due to orthodontic treatment procedure and poor periodontal status.

EXCLUSION CRITERIA

Grossly decayed teeth and attrited teeth were excluded because of the possibility of showing false positive results.

The study was divided into two groups: Group I (fresh teeth) comprised of 50 teeth which were analyzed within a period of a week. Group II (teeth kept for long period of time in soil) comprised of 50 teeth that were collected and kept for a period of 4-6 months and then analyzed

MATERIALS USED:

a) Extraction of teeth

Hand gloves

Mouth mask

Mouth Mirror

Probe

Kidney tray

Cotton rolls

Syringe

Disposable Needle

Local anesthetic gel

LA solution (lidocaine with adrenaline or without adrenaline)

Elevator

Extraction Forceps

b) Storing the teeth

Small containers

Soil

Adhesive Tape

Marker pen

c) Blood grouping

Sterile needle

Glass slide

Antiserum A and Antiserum B

d) Sectioning of teeth

Carborundum Disc

Straight fissure bur

Spoon excavator

Micromotor

e) Blood grouping from teeth

Test tubes

Test tubes racks

Slides

Cover slip

Cold physiologic saline

Bovine serum albumin

Antiserum A and antiserum B

Red blood cell suspension

Water bath

Centrifugal machine

Microscope

METHODOLOGY

A brief case history with relevant medical history was recorded from patients selected for study. The patients were informed about the study and consent was obtained. Following aseptic protocol the index finger was pricked with a sterile needle and two drops of blood was placed in a glass slide and to this antiserum -A and antiserum-B was added. If agglutination occurred when Antiserum-A was added the blood group belonged to group A and vice versa. If no agglutination occurs it belonged to O group and if agglutination occurs in both the drops then it s AB group. The obtained results were recorded and they were kept as gold standard. Then following local anesthetic administration extraction procedure was carried in aseptic protocol. The extracted teeth were washed under running water and a probe was used to remove blood, saliva or debris attached to it. The teeth were dried after wiping with gauze and placed in labeled small plastic container. From each tooth, the pulp was extirpated and the remaining tooth structure was pulverized in the following way. The tooth was completely trimmed to remove the enamel and cementum with lathe. The tooth was further split vertically with carborundum disc and the dental pulp was scooped out with

a spoon excavator. The remaining tooth consisted of dentin, which was pulverized, with straight fissure bur. Tooth pulp was divided into two equal parts and put into sterile labeled test tubes. The pulverized tooth powder of about 5-10 mg was put in two test tubes, to each of this test tubes 3 drops of antiserum A, B was added and confirming the test samples being sufficiently soaked with antiserum, the test tubes were plugged with cotton and it was places in a temperature of about 4-10^{0}C overnight to allow absorption. After removing antisera, each sample was washed 4-6 times with 10 ml ice cold saline solution in the following way. After addition of saline, the samples were agitated and then centrifuged for 5 min at 4,000 rpm. The supernatant was discarded using a Pasteur pipette and then excess saline was removed. Then two drops of 10% bovine serum albumin (BSA) was added to the samples and the test tubes were placed in a pre-heated water bath at 56°C for 10 min to elute the antibodies. The powder test samples containing dentin in BSA were centrifuged at 1,500-2,000 rpm for 1-2 min and then the supernatant was pipette and used for agglutination reaction. A drop of A and B red blood cell (RBC) suspensions which was prepared after centrifuging the blood samples, was added into the respective test tubes. Now, the samples were gently shaken and incubated at 37°C for 30 min to enhance agglutination process. After this, one drop of the solution was placed on a microscopic slide with a pipette then cover slip was placed, and finally agglutination was read microscopically at a magnification of ×10and ×40. And then it was cross confirmed with the blood groups which were already noted. The above mentioned procedure was done for Group I samples. Similar procedure was carried after 4-6 months after the day of extraction, in Group II samples which was kept in small labeled containers with soil and results were recorded.

FIGURE 1-ARMAMENTARIUM FOR EXTRACTION OF TEETH

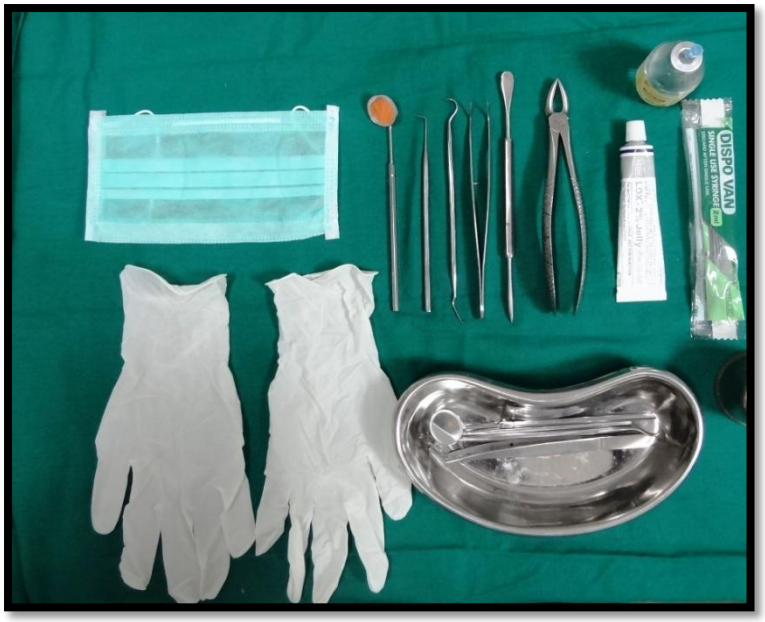


FIGURE 2-ARMAMENTARIUM FOR STORING THE TEETH

FIGURE 3-ARMAMENTARIUM FOR BLOOD GROUPING

FIGURE 4-ARMAMENTARIUM FOR SECTIONING THE TEETH

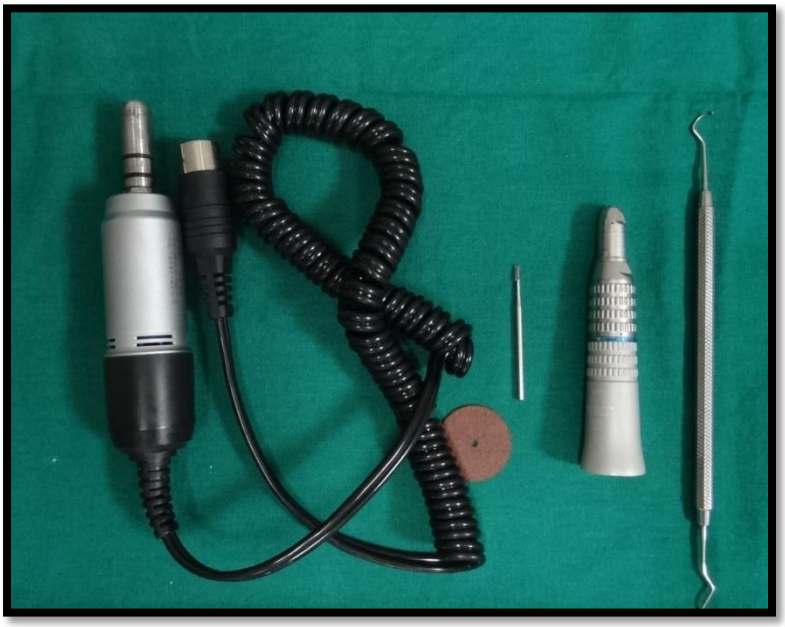


FIGURE 5-ARMAMENTARIUM FOR BLOOD GROUPING FROM TEETH

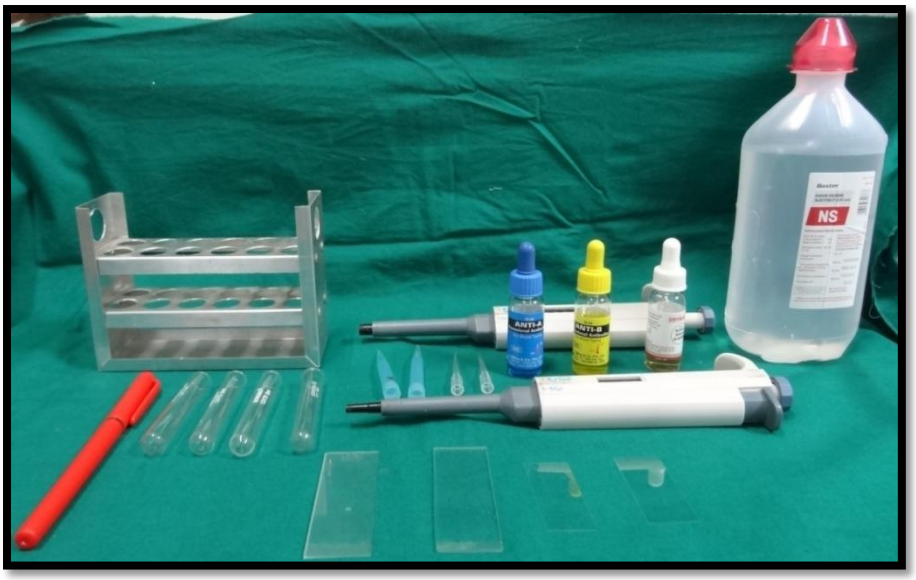
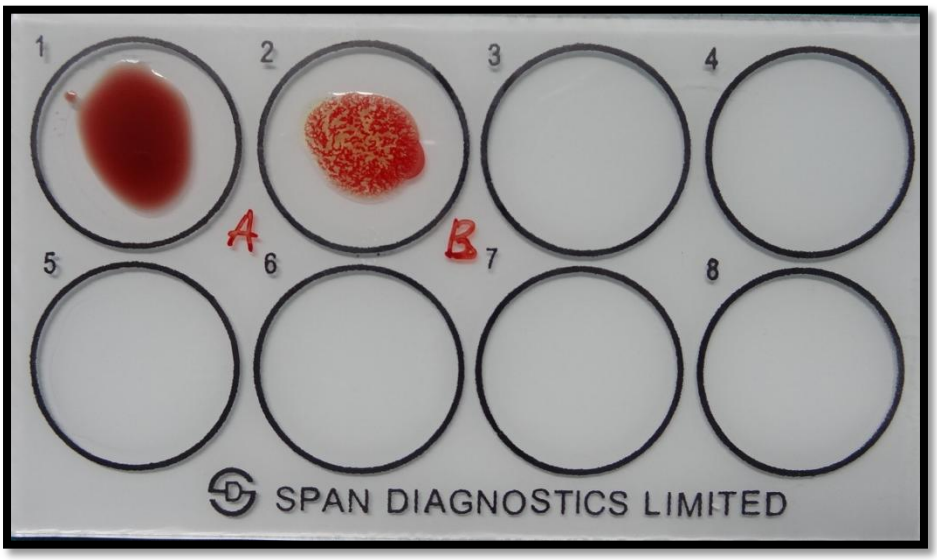


FIGURE 6- BLOOD GROUPING FROM PATIENT BY SLIDE AGGLUTINATION METHOD



TOOTH SECTIONING PROCEDURE

FIGURE 7, 8, 9- SHOWING SELECTED TEETH, SECTIONING USING CARBORANDUM DISC, SECTIONED TEETH RESPECTIVELY

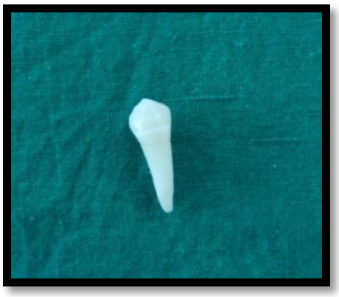


FIGURE-7

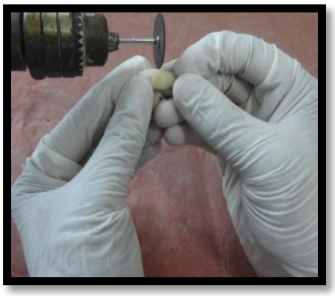


FIGURE-8

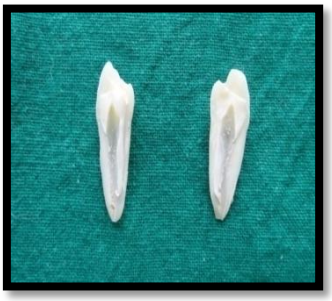


FIGURE-9

FIGURE 10, 11- SHOWING EXTRIPATED PULP AND ARROW INDICATES THE PULVARISED DENTIN RESPECTIVELY

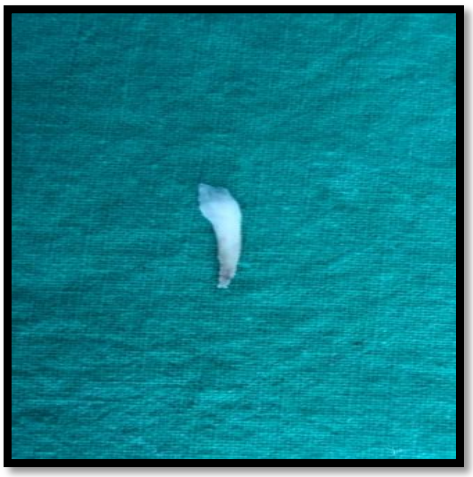


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FIGURE-10

FIGURE-11

BLOOD GROUPING FROM TEETH

FIGURE 12, 13, 14-SHOWING ANTI-SERA, IMMERSION OF PULP AND DENTIN IN ANTI-SERA AND CENTRIFUGATION RESPECTIVELY

FIGURE-12

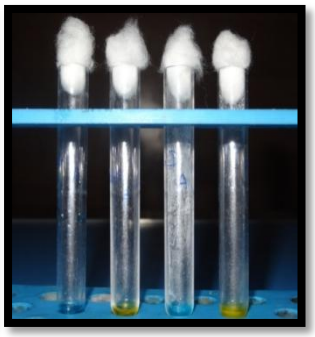

FIGURE-13

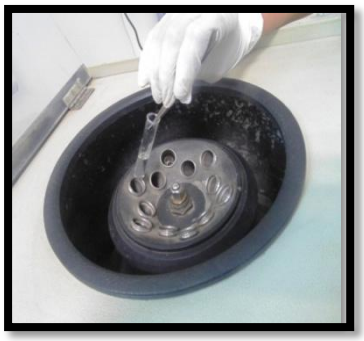

FIGURE-14

FIGURE 15,16,17-SHOWING ADDITION OF BOVINE SERUM ALBUMIN, AND IT IS KEPT IN WATER BATH AT 56^0 C

FIGURE-15

FIGURE-16

FIGURE-17

FIGURE 18 AND 19 SHOWING PREPARATION OF 0.5% RBC SUSPENSION

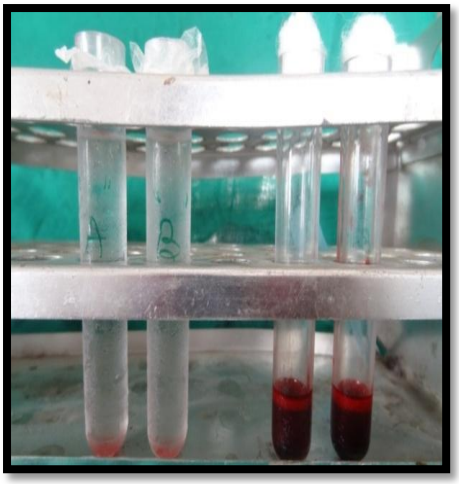

FIGURE-18

FIGURE-19

FIGURE -20 SHOWING PREPARED SLIDES

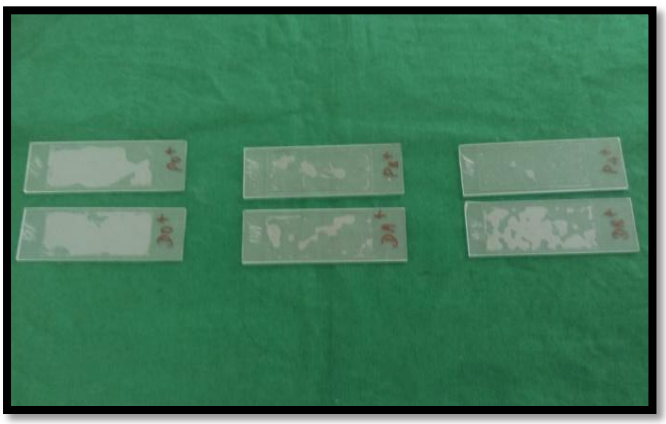

FIGURE-20

RESULTS

FIGURE 21 AND 22 SHOWING AGGLUTINATION IN PULP AND DENTIN RESPECTIVELY

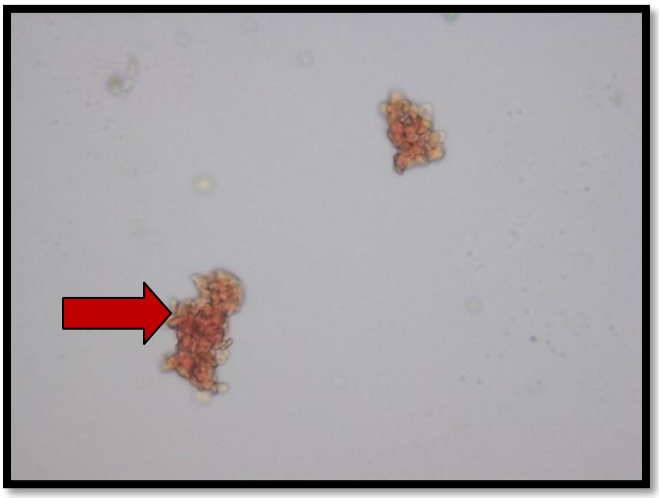

FIGURE 21-ARROW SHOWING AGGLUTINATION IN PULP

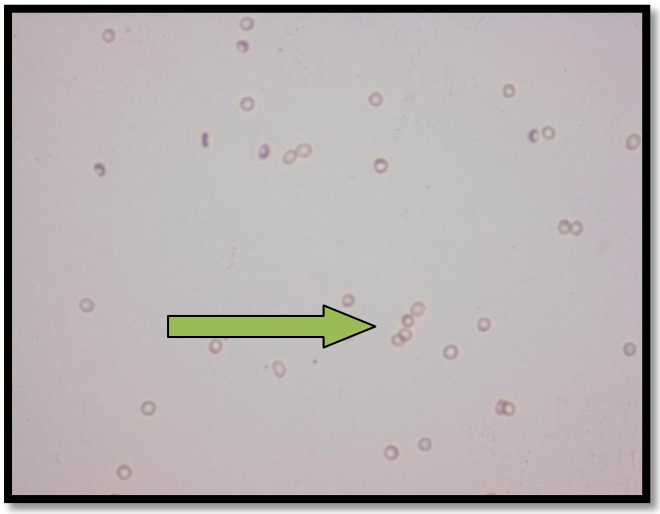

FIGURE 22-ARROW SHOWING AGGLUTINATION IN DENTIN

FIGURE 23& 24 SHOWING NO AGGLUTINATION IN PULP AND DENTIN RESPECTIVELY

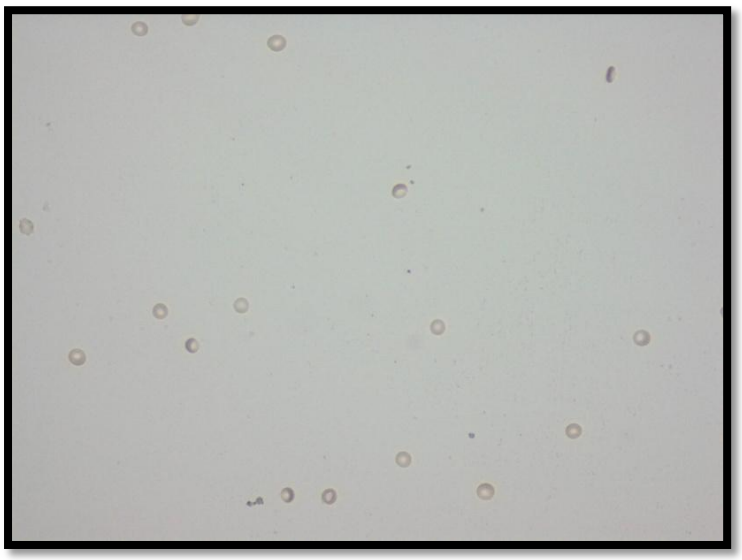

FIGURE-23-DISPERSED RED BLOOD CELLS

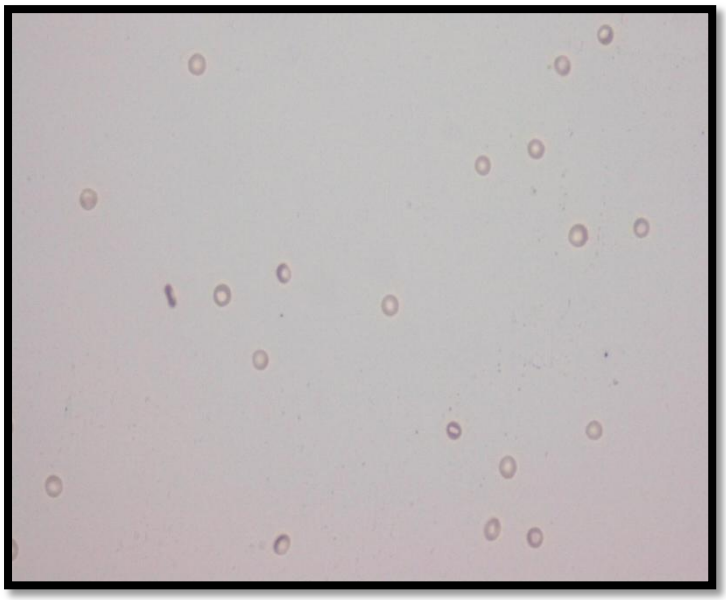

FIGURE 24-NO AGGLUTINATION

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Data obtained was analyzed using Statistical package for Social Sciences (SPSS) software version 16.0 (Windows version 17.0; SPSS Inc., Chicago, IL, USA). Intra and intergroup analysis was done using Pearson's Chi square test. In the present study, the level of significance (α) was fixed at 5%. ($p \leq 0.05$).

For calculating the test statistic:-

The value of the test-statistic is

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} = N \sum_{i=1}^{n} p_i \left(\frac{O_i/N - p_i}{p_i} \right)^2$$

where

χ^2 = Pearson's cumulative test statistic, which asymptotically approaches a χ^2 distribution.

O_i = the number of observations of type *i*.

N = total number of observations

$E_i = Np_i$ = the expected (theoretical) frequency of type *i*, asserted by the null hypothesis that the fraction of type *i* in the population is p_i

n = the number of cells in the table

Sensitivity between the test group and reference was analysed. The results were obtained in percentage. Both intra group analysis and inter-group analysis was done. Intra group analysis was done between all the parameters like age, sex, type of blood group and the teeth analyzed.

Cramer's V and Phi correlation co-efficient was employed to measure correlation between the test group and the reference since the data obtained was nominal.

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RESULTS

RESULTS

Hundred teeth were collected from patients in the age group of 11-70 years. The blood group was determined by slide agglutination method and was taken as reference. Then the teeth were divided into two groups where in Group I (n=50) the procedure was done immediately after extraction and the results were obtained. In group II (n=50) the procedure was done 4-6 months after extraction where the samples were stored in soil. When blood grouping from the pulp and hard tissue correlated with blood group of person which was identified from the reference, the test result was recorded as "positive" and if they did not match, then it was recorded as "negative. The working results which was obtained is as shown in Table I and II

TABLE-1 ILLUSTRATING THE RESULTS OBTAINED FROM STUDY IN GROUP I

S.No	Age (In Yrs)	Sex	Reference Blood Group	Tooth	Pulp	Dentin	Teeth
1	50	M	O	16	Positive	Positive	Positive
2	60	M	A	18	Positive	Negative	Negative
3	51	F	AB	25	Positive	Positive	Positive
4	42	M	AB	46	Negative	Positive	Negative
5	32	M	B	36	Positive	Positive	Positive
6	26	M	A	46	Positive	Positive	Positive
7	34	M	AB	36	Positive	Positive	Positive
8	23	M	A	27	Positive	Positive	Positive
9	54	F	O	21	Positive	Positive	Positive
10	26	M	B	36	Positive	Positive	Positive
11	42	M	B	23	Positive	Positive	Positive
12	47	F	A	26	Positive	Positive	Positive
13	51	M	A	25	Positive	Negative	Negative
14	37	F	O	37	Positive	Positive	Positive
15	41	F	A	18	Negative	Negative	Negative
16	48	F	O	26	Positive	Positive	Positive
17	21	M	B	15	Positive	Positive	Positive
18	18	M	A	14	Positive	Positive	Positive
19	27	F	A	18	Positive	Positive	Positive
20	44	M	AB	25	Positive	Negative	Negative
21	17	M	A	24	Negative	Positive	Negative

22	36	M	B	36	Positive	Positive	Positive
23	14	M	O	24	Positive	Positive	Positive
24	14	M	O	24	Positive	Positive	Positive
25	48	F	AB	45	Positive	Positive	Positive
26	19	M	O	14	Positive	Positive	Positive
27	15	F	O	14	Positive	Positive	Positive
28	17	M	O	24	Positive	Positive	Positive
29	66	F	A	11	Positive	Positive	Positive
30	15	F	B	14	Positive	Positive	Positive
31	50	M	A	14	Positive	Negative	Negative
32	54	F	O	34	Positive	Negative	Negative
33	19	M	O	24	Positive	Positive	Positive
34	50	M	A	15	Negative	Negative	Positive
35	53	F	B	34	Positive	Positive	Positive
36	13	M	A	14	Positive	Positive	Positive
37	38	F	B	12	Positive	Positive	Positive
38	11	F	O	14	Positive	Positive	Positive
39	38	F	B	13	Positive	Positive	Positive
40	15	F	B	24	Positive	Positive	Positive
41	54	F	O	34	Positive	Negative	Negative
42	19	M	O	35	Positive	Positive	Positive
43	53	F	B	35	Positive	Negative	Negative
44	66	F	A	12	Positive	Positive	Positive
45	15	F	B	34	Positive	Positive	Positive
46	11	F	O	24	Positive	Positive	Positive
47	13	M	A	44	Positive	Positive	Positive
48	19	M	O	44	Positive	Positive	Positive
49	15	F	B	44	Positive	Positive	Positive
50	18	F	O	24	Positive	Positive	Positive

MALE-M FEMALE-F

TABLE II-ILLUSTRATING THE RESULTS OBTAINED FROM STUDY IN GROUP II

S.No	Age (In Yrs)	Sex	Reference Blood Group	Tooth	Pulp	Dentin	Teeth
1	14	M	O	14	Positive	Negative	Negative
2	14	M	A	14	Positive	Positive	Positive
3	28	F	B	28	Positive	Positive	Positive
4	14	F	B	14	Positive	Positive	Positive
5	65	F	A	23	Negative	Negative	Negative
6	15	F	O	14	Negative	Negative	Negative
7	14	M	O	24	Positive	Negative	Negative
8	11	F	B	25	Positive	Positive	Positive
9	14	M	O	24	Positive	Positive	Positive
10	50	F	B	43	Positive	Negative	Negative
11	67	F	B	44	Positive	Negative	Negative
12	19	F	B	14	Positive	Positive	Positive

13	14	M	A	44	Positive	Positive	Positive
14	23	M	B	14	Positive	Positive	Positive
15	14	M	O	34	Positive	Negative	Negative
16	12	F	B	14	Positive	Positive	Positive
17	32	F	A	34	Negative	Negative	Negative
18	23	M	B	14	Positive	Positive	Positive
19	14	F	B	24	Positive	Positive	Positive
20	15	F	O	25	Negative	Negative	Negative
21	22	M	AB	14	Positive	Positive	Positive
22	51	F	AB	11	Negative	Negative	Negative
23	23	M	B	24	Positive	Positive	Positive
24	14	M	O	44	Positive	Negative	Negative
25	23	M	B	24	Positive	Negative	Negative
26	67	F	B	43	Negative	Negative	Negative
27	14	M	O	34	Positive	Negative	Negative
28	19	F	B	44	Positive	Negative	Negative
29	14	F	B	34	Positive	Negative	Negative
30	22	M	AB	24	Positive	Positive	Positive
31	16	F	O	24	Positive	Positive	Positive
32	22	M	AB	34	Positive	Negative	Negative
33	54	F	B	11	Positive	Negative	Negative
34	15	F	O	34	Negative	Negative	Negative
35	12	F	B	45	Positive	Negative	Negative
36	23	M	B	34	Positive	Positive	Positive
37	11	F	B	35	Positive	Positive	Positive
38	23	M	B	34	Positive	Positive	Positive
39	50	F	B	34	Positive	Positive	Positive
40	17	M	O	24	Positive	Positive	Positive
41	23	M	B	44	Positive	Negative	Negative
42	14	F	B	44	Positive	Negative	Negative
43	16	F	O	24	Positive	Positive	Positive
44	65	F	A	24	Negative	Negative	Negative
45	51	F	AB	13	Negative	Negative	Negative
46	23	M	B	44	Positive	Negative	Negative
47	50	F	B	35	Positive	Negative	Negative
48	22	M	AB	44	Positive	Negative	Negative
49	15	F	O	44	Negative	Negative	Negative
50	51	F	AB	14	Negative	Negative	Negative

MALE-M FEMALE-F

DESCRIPTIVE ANALYSIS

The study comprises of 26 males and 24 females in group I and 21 males and 29 females in group II (Table IV). The age groups were divided into 6 subgroups where in group I 19 patients were in age group of 11-20, 5 patients 21-30yrs, 6 patients 31-40 yrs, 9 patients 41-50 yrs,9 patients 51-60 yrs 2 patients 61-70 yrs. In group II 25 patients were in age group of 11-20, 13patients 21-30yrs, 1 patient 31-40 yrs, 3 patients 41-50 yrs, 4 patients in 51-60 yrs 4 patients 61-70 yrs. (Table III). The teeth which were taken for analysis in group I was 33 maxillary teeth and 27 mandibular teeth. In group II it was 27 maxillary teeth and 23 mandibular teeth. (Table V). The distribution of blood group is as follows, 15 patients belonged to A, 13 patients belonged to B, 5 patients belonged to AB, 17 patients belonged to O in group I and 5 patients belonged to A, 24 patients belonged to B, 8patients belonged to AB 13 patients belonged to O in group II (Table VI)

TABLE III- AGE DISTRIBUTION OF GROUP I AND GROUP II

	AGE (IN YRS) (n=50)					
	11-20	**21-30**	**31-40**	**41-50**	**51-50**	**61-70**
GROUP I	19(38%)	5(10%)	6(12%)	9(18%)	9(18%)	2(4%)
GROUP II	25(50%)	13(26%)	1(2%)	3(6%)	4(8%)	4(8%)

TABLE IV- SEX DISTRIBUTION IN GROUP I AND GROUP II

SEX DISTRIBUTION (n=50)		
	MALES	**FEMALES**
GROUP I	26(52%)	24(48%)
GROUP II	21(42%)	29(58%)

TABLE V- JAW DISTRIBUTION IN GROUP I AND GROUP II

JAW DISTRIBUTION (n=50)		
	MAXILLARY	**MANDIBULAR**
GROUP I	33(66%)	27(34%)
GROUP II	27(54%)	23(46%)

TABLE VI- BLOOD GROUP DISTRIBUTION IN GROUP I AND GROUP II

BLOOD GROUP DISTRIBUTION (n=50)				
	A	**B**	**AB**	**O**
GROUP I	15(30%)	13(26%)	5(10%)	17(34%)
GROUP II	5(10%)	24(48%)	8(16%)	13(26%)

RESULTS

TABLE VII- PERFORMANCE OF PULP, DENTIN AND TEETH IN RELATION TO REFERENCE SAMPLE

CORRELATION WITH CONTROL	GROUP I		GROUP II	
	POSITIVE	PICK-UP RATE (%)	POSITIVE	PICK-UP RATE (%)
PULP	46	92.0	39	78.0
DENTIN	41	82.0	21	42.0
TEETH (PULP+ DENTIN)	39	78.0	21	42.0

Table VII and graph I shows the comparison of pulp dentin and teeth with the reference group. In Group I (n = 50), when pulp was compared with the reference, 46 teeth showed positive results, while four were negative. The sensitivity of pulp in comparison to blood was found to be 92%. Dentin when compared with blood, 41 teeth showed positive results and the sensitivity was 82% .Teeth (pulp +dentin) correlated only in 39 samples with a sensitivity of 78%. In Group II (n = 30), for pulp, 39 samples showed positive results and eleven showed negative result with a sensitivity of 78%. Comparison of dentin with blood showed 21 samples correlating reference group with a sensitivity of 42%.Complete correlation for teeth was seen in only 21 samples with 29 negative results showing a sensitivity of 42%

TABLE VIII- PERFORMANCE OF PULP AND DENTIN IN VARIOUS AGE DISTRIBUTIONS OF GROUP I

AGE IN YEARS	TOTAL	PULP (%)	DENTIN (%)
11-20	19	18 (94.7)	18 (94.7)
21-30	5	5 (100)	5 (100)
31-40	6	6 (100)	6 (100)
41-50	9	7(77.7)	6(66.7)
51-60	9	8 (88.9)	3(33.3)
61-70	2	2 (100)	2 (100)
p-value		0.562	0.002

Table VIII and graph II shows the comparison of pulp and dentin in various age distributions of group I, where 100 % the findings were positive for pulp and dentin among the age group of 21-30, 31-40 and 61-70. There is some variation in the results in the age group of 51-60. The association with age group and the pulp is statistically insignificant with p-value- 0.562 ($p< 0.05$). The association with age group and dentin is statistically significant with p-value- 0.002 ($p< 0.05$).

TABLE IX - PERFORMANCE OF PULP AND DENTIN IN VARIOUS AGE DISTRIBUTIONS OF GROUP II

AGE IN YEARS	TOTAL	PULP (%)	DENTIN (%)
11-20	25	21 (84)	12 (48)
21-30	13	13(100)	8 (61.5)
31-40	1	0	0
41-50	3	3(100)	1 (33.3)
51-60	4	1(25)	0
61-70	4	1(25)	0
p-value		0.001	0.108

Table IX and graph III shows the comparison of pulp and dentin in various age distributions in group II, where in age group 31-40 both pulp and dentin gave negative results when compared to reference groups. In patients ranging from age group 51-70 the dentinal tissues did not give any positive results with the reference group. The

association with age group and the pulp is statistically significant with p-value- 0.001 ($p< 0.05$). The association with age group and dentin is statistically insignificant with p-value- 0.108 ($p< 0.05$).

TABLE X- PERFORMANCE OF PULP AND DENTIN OF MALES AND FEMALES IN GROUP I

SEX	TOTAL NUMBER	PULP (%)	DENTIN (%)
MALE	26	23 (88.5)	20 (76.9)
FEMALE	24	23 (95.8)	20 (83.3)
p-value		0.337	0.571

Table X and graph IV shows that in group I pulp tissue gave positive results in 85.5% males and 95.8% females where as in dentin the results was positive for 76.9 % males and 83.3 % females. Since the distribution of data is wide the p-value ($p<0.05$) is not statistically significant.

TABLE XI- PERFORMANCE OF PULP AND DENTIN OF MALES AND FEMALES IN GROUP II

SEX	TOTAL NUMBER	PULP (%)	DENTIN (%)
MALE	21	21 (100)	11 (52.3)
FEMALE	29	18 (62.0)	10 (34.4)
p-value		0.001	0.206

Table XI and graph V shows that in group II pulp tissue gave positive results in 100% of male and 62% females where as in dentin the results was positive for 52.3 % males and 34.4% females. The association of male and female with the results from the pulp gave a statistically significant p- value 0.001 ($p<0.05$). The association of male and female with the results from dentin showed statistically insignificant p- value 0.206 ($p<0.05$)

TABLE XII- PERFORMANCE OF PULP AND DENTIN OF MAXILLARY AND MANDIBULAR ARCH IN GROUP I

TEETH	TOTAL NUMBER	PULP (%)	DENTIN (%)
MAXILLARY	33	30(90.9)	26(78.8)
MANDIBULAR	17	16(94.1)	14(82.3)
p-value		0.692	0.765

Table XII and graph VI shows that in group I the mandibular teeth are good reservoirs to store the pulp and dentin tissues where the blood group antigens are protected in about 94.1% in pulp tissues and 82.3% in dentinal tissues. Since the results do not show much variation the association of arches with that of dentin and pulp is statistically not significant.

TABLE XIII- PERFORMANCE OF PULP AND DENTIN OF MAXILLARY AND MANDIBULAR ARCH IN GROUP II

TEETH	TOTAL NUMBER	PULP (%)	DENTIN (%)
MAXILLARY	27	20 (74.0)	16 (59.5)
MANDIBULAR	23	19 (82.6)	5 (21.7)
p-value		0.468	0.007

Table XIII and graph VII shows that in group II the pulp of mandibular teeth showed 82.2 % positive results whereas the dentin of maxillary teeth showed 59.5% positive results. So, with respect to group II, the maxillary teeth are superior to protect the blood group antigens in dentin especially after about 4-6 months of investigations .The association of arches with that of dentin and pulp is statistically not significant. ($p<0.05$).

TABLE XIV-COMPARISON OF INDIVIDUAL BLOOD GROUPS WITH REFERENCE IN PULP AND DENTIN –GROUP I

BLOOD GROUP	NUMBER	PULP (%)	DENTIN (%)
A	15	12(80)	9(60)
B	13	13(100)	12(92.3)
AB	5	4 (80)	4 (80)
O	17	17(100)	15 (88.2)
p value		0.089	0.127

Table XIV and graph VIII shows that B antigens in pulp (100%) and dentin (92.3%) showed excellent results. Followed by this, AB antigens were detected in 80% of the cases. Since the results do not show much variation the association of blood group with dentin and pulp is not statistically significant. ($p<0.05$)

TABLE XV-COMPARISON OF INDIVIDUAL BLOOD GROUPS WITH REFERENCE IN PULP AND DENTIN –GROUP II

BLOOD GROUP	NUMBER	PULP (%)	DENTIN (%)
A	5	2(40.0)	2(40.0)
B	24	24(100)	13(54.2)
AB	8	4(50)	2(25)
O	13	9(69.2)	4(30.8)
P value		0.002	0.378

Table XIV and graph VIII shows that the B antigens were detected in all the cases from the pulp even if it was processed after 4-6 months. Blood group antigens were detected from dentin in 40% of cases with A antigen, 54.2% with B antigen, 25% with AB antigen and 30.8% with O antigen. The association of pulp with blood group antigens was statistically significant p-0.002 ($p<0.05$)

CORRELATION CO-EFFICIENT

TABLE XVI-CORRELATION OF PULP AND HARD TISSUE IN RELATION TO REFERENCE USING CRAMER'S V CORRELATION CO-EFFECIENT

	CRAMER'S V CO-EFFICIENT (*V*)	**p * VALUE**
GROUP I		
Pulp vs. reference	0.361	0.089
Dentin vs. reference	0.338	0.127
Both vs. reference	0.357	0.095
GROUP II		
Pulp vs. reference	0.552	0.004
Dentin vs. reference	0.249	0.378
Both vs. reference	0.249	0.378

*- Pearson Chi square test

TABLE XVII-CORRELATION OF PULP AND DENTIN IN GROUP I AND GROUP II WITH PHI CORRELATION CO-EFFICIENT

GROUP I	**PHI COEFFICIENT**	**p * VALUE**
Pulp vs. Dentin	0.405	0.004
GROUP II		
Pulp vs. Dentin	0.452	0.004

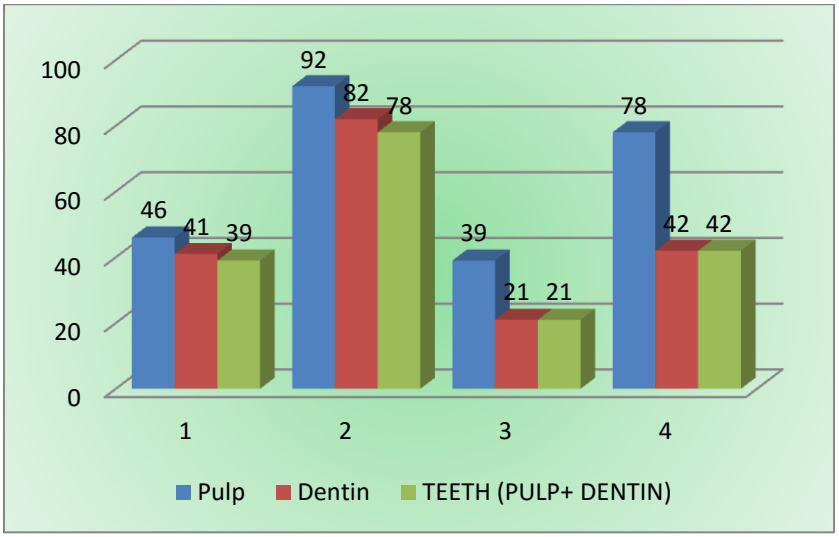
*- Pearson Chi square test

Cramer's V and Phi correlation co-efficient is employed to measure correlation between two groups working with nominal data. When the data matrix is 2x2, the Phi statistic is used. Cramer's V is used when the number of rows and columns is unequal (2x3, 3x5, 5x7).The values between 0.1 -0.3 indicate small correlation. In the analysis of pulp and dentin against the reference samples, a weak correlation was seen in group I while in group II pulp sample suggest moderate correlation (Cramer's V co-efficient (V =0.552) which was statistically significant (p =.004). By combining pulp and hard tissue in both the groups the correlation is moderate (Table-XVI)

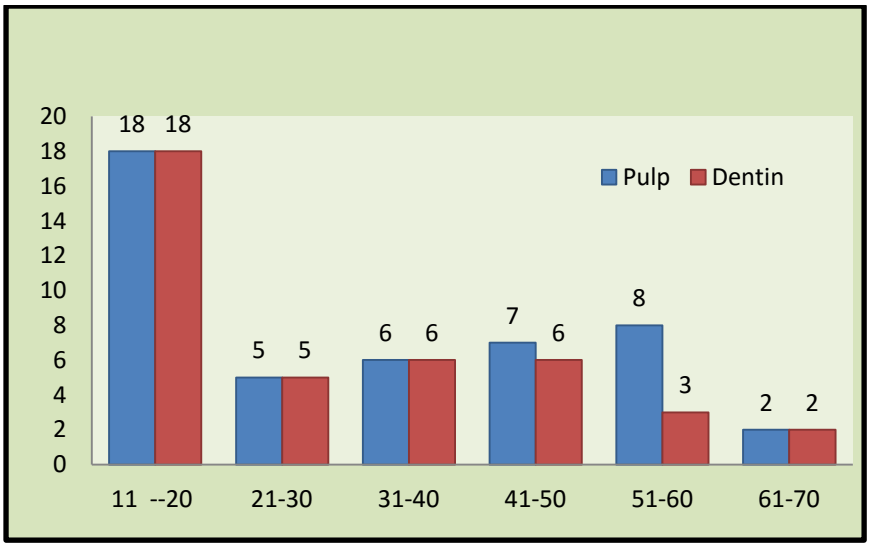
By combining pulp and hard tissue in both the groups the correlation is moderate with Phi coefficient of 0.452 (Table-XVII).

GRAPHS

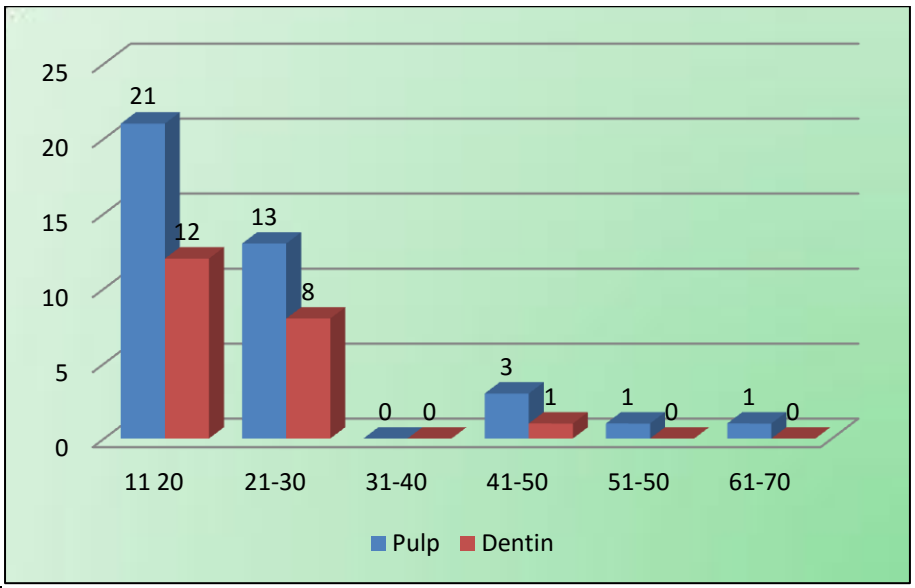
Graph I- Performance of Pulp, Dentin and Teeth in Relation to Reference Sample



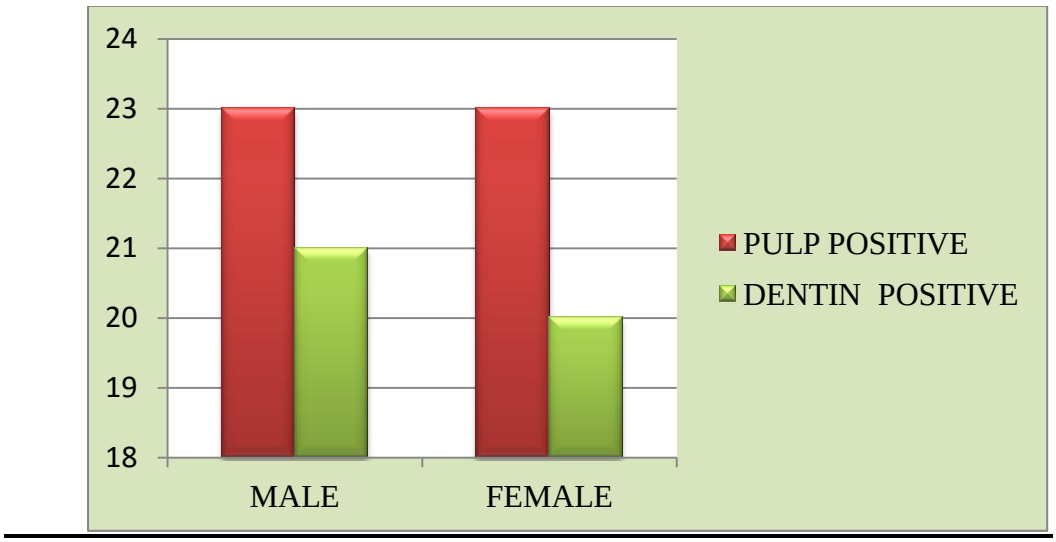
Graph II- Performance of Pulp and Dentin of Various Age Distributions in Group I



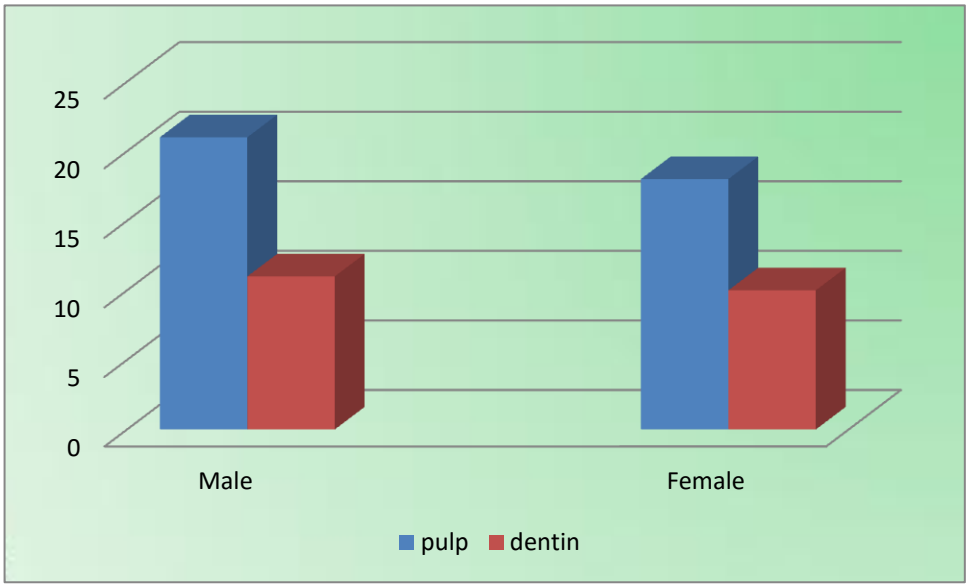
Graph III- Performance of Pulp and Dentin of Various Age Distributions in Group II



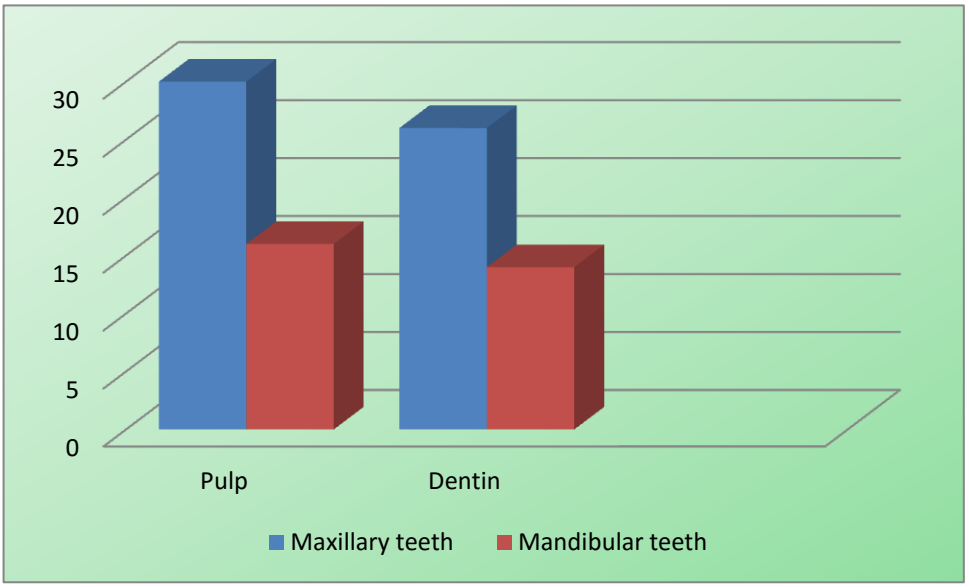
Graph IV- Performance of Pulp and Dentin of Males and Females in Group I



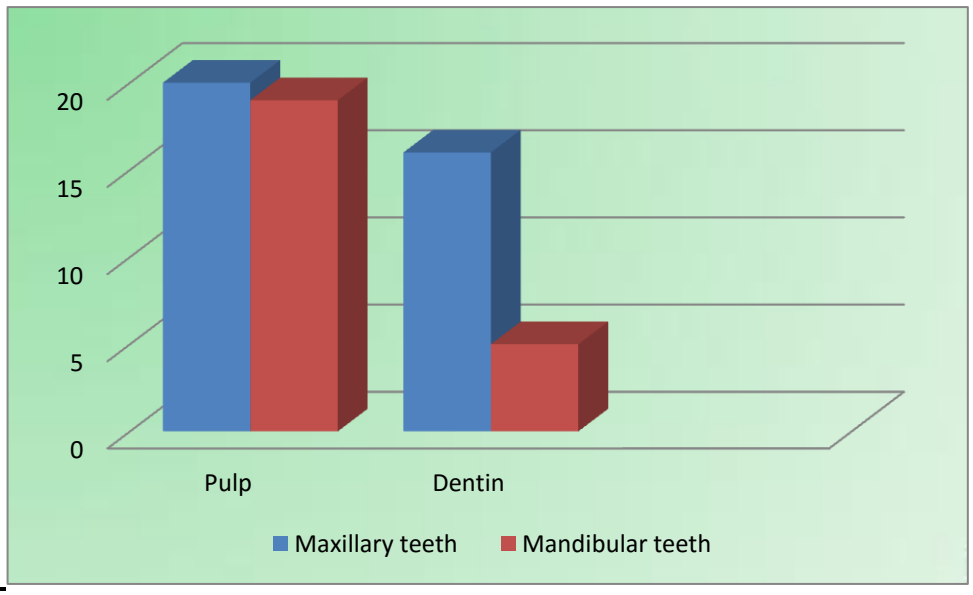
Graph V- Performance of Pulp and Dentin of Males and Females in Group II



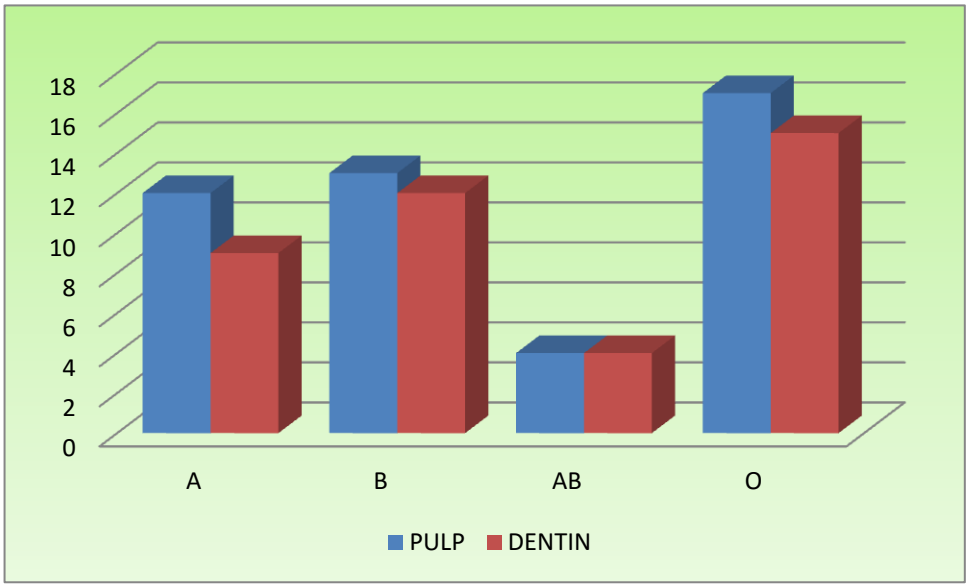
Graph VI- Performance of Pulp and Dentin of Maxillary and Mandibular Arches in Group I



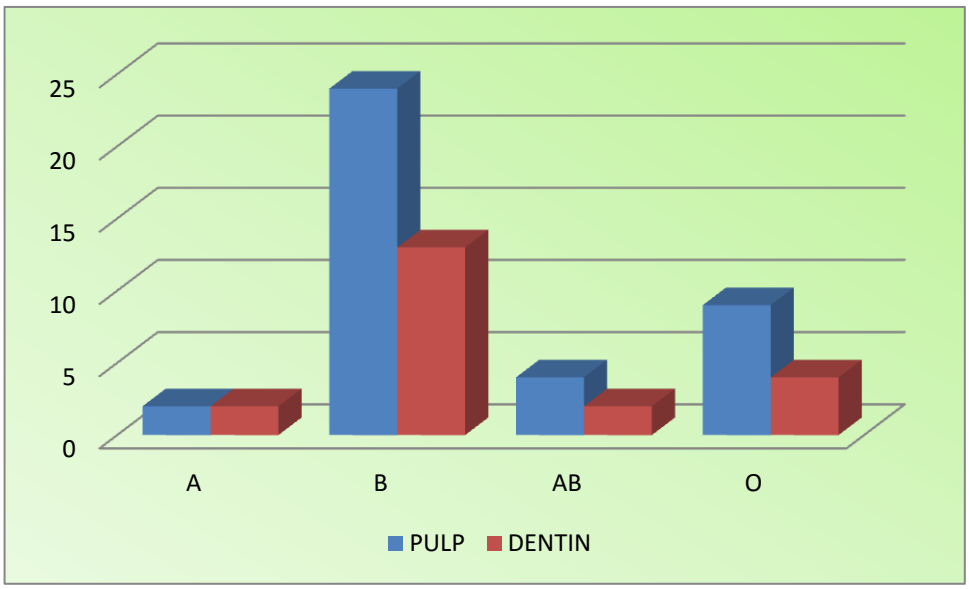
Graph VII- Performance of Pulp and Dentin of Maxillary and Mandibular Arches in Group II



Graph VIII-Comparison of Individual Blood Groups with Reference in Pulp and Dentin –Group I



Graph IX-Comparison of Individual Blood Groups with Reference in Pulp and Dentin –Group II



DISCUSSION

DISCUSSION

In Forensic Science, identification of a person is based on comparison between known characteristics of a missing individual collected previously, (termed as ante mortem data) with recovered characteristics from an unknown body, (termed post-mortem data). Being diverse and resistant to environmental challenges, teeth are considered excellent post-mortem material for identification with enough concordant points to make a meaningful comparison[29.]

Over the past few decades, information from studies on blood groups has been applied to medico-legal application. The use of blood group substances plays a significant role in identification of a person. This is based on the fact that once a blood group is established in an individual, it remains unchanged throughout the life.[30]

There are various blood grouping systems, in which the ABO system is the most reliable and oldest which plays an important role in transfusion medicine. This was discovered by Austrian scientist, Karl Landsteiner, who found three different blood types (A, B and O) in 1900 from serological differences in blood called the Landsteiner Law. In 1902, Des Casterllo and Sturli discovered the fourth type, AB. The ABO blood group is determined by the presence of A and B antigens on the surface of the red blood cells, and of anti -A or anti -B antibodies in the serum. Thus, the red blood cells of blood type A possess antigen A and the serum containing anti –B antibody. Similarly, blood type B has antigen B and anti -A antibody. Blood type AB contains both A and B antigens but no antibodies. Blood type O has no antigens but contains both anti -A and anti –B antibodies. Anti -A and anti -B antibodies are usually IgM type, and not present in newborns, but appear in the first year of life[31].

Out of various tissues in the human body, dental enamel is the hardest which can withstand peri and post-mortem damages. Followed by enamel is the dentin which is stronger than bone but less strong than enamel. The pulp which is enclosed by these hard tissues is well protected which carries the ABO blood group antigens in a comparatively larger proportion than dentin and enamel.[29]

In the present study hundred teeth were collected and were divided into two groups. In group I with 50 teeth the entire procedure of determining the blood group was done within few days of extraction. In group II the procedure was done 4-6 months after the extraction procedure. This was stored in soil. Soil environment is a gestalt of many factors. Some factors are abiotic and some are biotic. There are various factors contributing to the soil ecosystem as soil water, absorptive capacities of clay minerals, temperature, pH, soil atmosphere oxidation-reduction potential and various complex reactions due to the microorganisms[32].

It is possible that the antibodies are produced against certain food substances and environmental antigens (bacterial, viral or plant antigens), which are similar in structure to A and B antigens. Considering this fact, the teeth in group II were stored in soil to see any changes that may interfere in blood grouping[31].

The extensive inorganic content in teeth made it difficult to determine the blood grouping by absorption-inhibition method which was formerly used by various forensic scientists. As this method required decalcification procedures and also results were not reliable, other methods like absorption elution and haemagglutination were employed [11]. The current choice for typing of dried blood stains is absorption elution technique. Various studies have obtained positive results for blood grouping on teeth using this technique [3,10,23,27]. Also a study was conducted by **Nishi et al in 2005[33]**, where

the thermostability of blood group antigens were analyzed in severely burned bodies by absorption elution technique and the results were remarkable.

The principle of absorption elution test lies in the absorption of blood group specific antibodies on the surface of a substance having blood group antigens, elution of the antibody so absorbed under a high temperature, and the agglutination of the blood cells possessing corresponding antigens. Also, the surface area of the material used becomes important. Hence, the hard tissue of teeth is pulverized to fine powder to increase the surface area for reaction. In our study, absorption of antibodies was facilitated by placing the test samples in antisera overnight at 4°C. Placing the samples in this temperature and later washing the samples at the same temperature increases agglutination intensities.[34].Following this, bovine serum albumin was added to increase the sensitivity of the reagents. This is achieved by the action of albumin which may neutralize ionic repulsion between erythrocytes and enhance specific linkage between corresponding antigen and antibodies. They are also found to block non-specific binding of antibodies on to the specimen[25].Before adding the red cell suspension, the test samples containing hard tissue powder are centrifuged at 1,200-1,500 rpm for 1-2 min. The supernatant is then decanted and sucked and to this, the red cell suspension is added. This step is done to avoid the interference of hard tissue powder with reading of the agglutination results [3.]

In this study hundred teeth were collected from patients in the age group of 11-70 years which were extracted due to periodontal diseases and for orthodontic treatment. Carious teeth were excluded from the study as it may give false-positive results. The blood group was determined by slide agglutination method and was taken as reference. In the first group the procedure was done immediately after

extraction and the results were obtained. In second group the procedure was done 4-6 months after extraction where the samples were stored in soil.

In Group I, when pulp was compared with the reference, 46 teeth showed positive results, while four were negative. The sensitivity of pulp in comparison to blood was found to be 92%. In other similar studies by **Lele [35](1970), Garg and Garg[36](1989), Smeets et al[6] (1991) Sharma and Chattopadhyay[23] (1993), Ballal and David[10] (2011), Aswath, et al[27] (2012), Ramnarayan BK et al[3] (2013), Saloni Sood [37](2015)** have shown a sensitivity of 86%,92%,80%,100%,90%,95%93%,89% respectively. Dentin, when compared with blood, 41 teeth showed positive results and the sensitivity was 82%. In similar studies by **Ramnarayan BK et al[3] (2013),** the sensitivity was 86%. Since pulp contains a lot of blood vessels, these antigens are almost certainly bound to be present in tooth pulp. Hence, determination of blood group from pulp is relatively simple (Smeets et al[6]). This has been confirmed in the other similar studies which have been mentioned previously. The major accepted theory for the origin of blood antigens in dental hard tissue is based on infusion sedimentation phenomenon combined with inherently present antigen. This theory describes the infusion of water soluble antigens from saliva into the tooth surface[11].It is also presumed that blood group antigens are present in dentinal tubules. Studies by Nakayama et al[24] have shown that the odontoblast including the dentinal process are one of the positive sources of blood group antigenicity and also the blood group activity of the tooth fragment is markedly decreased when the odontoblastic zone is scrapped off.

In Group II for pulp, 39 samples showed positive results and eleven showed negative result with a sensitivity of 78%. Similar studies by **Smeets et al [6](1991) Sharma and Chattopadhayay[23] (1993)**, **Shetty et al[26] (2011),** and **Ramnarayan BK et al[3] (2013),** showed 86%, 100%, 96.7% and 83.3%, sensitivity respectively. The negative

results of pulp for blood grouping may be attributed to insufficient quantity of pulp, fibrosis of pulp tissue with increasing age, increased calcification of the canal and lysis of the cell. In the present study only 25% of the patients showed positive results in the age of 50 yrs and above. The negative results may be due to certain changes in the pulp tissue and also due to the contamination of the samples. Comparison of dentin with blood showed 21 samples correlating with the reference group and a sensitivity of 42% was determined. In a similar study conducted by **Shetty et al[26] (2011), Ramnarayan BK et al[3] (2013),** the sensitivity was 0 and 76.7% respectively**.** The negative results may be due less amount of antigens in the hard tissue, failure of technique and contamination of the sample

Complete correlation of soft and hard tissue was seen in 39 samples in group I with sensitivity of 78% and in group II it was 42%. In a similar study by **Ramnarayan BK et al[3] (2013),** the overall correlation was 73.3% and 66.6%. In both the studies bovine serum albumin was used to increase the sensitivity of the reagent and hence agglutination could be seen well.

In this study, considering age as a parameter, the results obtained showed a gradual decrease in sensitivity (%) when compared to the control. The distribution of number of samples in each sub-group was not equal. The percentage obtained was compared with the control. As age progresses the volume of pulp is decreased due to fibrosis or increased calcification of canal[35].

In this study, the pulp and hard tissue of female patients showed better results than that of males in group I. Whereas, in group II results were superior for the male patients compared to female patients. This variation is mainly attributed due to the differences in the number of samples selected for the study. However, studies by Garg and Garg[36], Lele et al.[35], show no sex difference in their result

Pulp and hard tissue of mandibular teeth showed good results in group I, but in group II, mandibular teeth showed better results .Whereas, the maxillary teeth showed superior results when compared to the control. This difference may be due to the amount of pulp tissue present and also the bulk of tooth which may protect the antigens present in the pulp. Studies by **Lele et al**[35] and **Ramnarayan BK et al**[3] showed better results in the maxillary teeth.

When individual blood group was analyzed in group I for pulp tissue, B and O group showed complete correlation. In similar studies by **Aswath et al**[27] and **Ramnarayan BK et al**[3] A, B ,O in former study and B group in latter study showed complete correlation .In group II complete correlation was seen in B group, followed by that is the O group (69.2%). In case of dentin, with respect to both the groups, no blood group was correctly matched with the reference group. In the present study no false results were obtained. False results may be mainly attributed due to contamination or due to acquired antigen[22].

Cramer's V and Phi correlation co-efficient is employed to measure correlation between two groups working with nominal data. The values between 0.1 -0.3 indicate small correlation. In the analysis of pulp and dentin against the reference samples, a weak correlation (V=0.361) was seen in group I while in group II pulp sample suggest moderate correlation (V=0.552) .By combining pulp and hard tissue in both the groups the correlation was moderate in both the groups. The present study could not be compared with other studies with respect to correlation co-efficient because other correlation co-efficient test was applied in those studies. In a study conducted by **Ramnarayan BK et al**[3]**,** Goodman-Kruskal's gamma co‑efficient was done to see the correlation where, pulp showed large correlation in freshly extracted teeth and the correlation was moderate in another group where it was stored for 6 months.

Pearson Chi Square Test indicates that there was no significant difference in the blood groupings between two methods: Slide agglutination and modified absorption elution. It was assumed that there is no difference in the blood groupings between two methods. The assumption of no difference in null hypothesis was formulated for the present study and this hypothesis was tested by Pearson Chi square test .Null hypothesis has to be rejected, if P value of the test statistic is < 0.05. In the present study P value of 0.089 (P = 0.083 was > 0.05) was obtained, for pulp vs. reference in group I and for dentin vs. reference in group I it was 0.127. Hence it is inferred that the blood group obtained by slide agglutination method and blood group obtained by absorption elution method are the same in group I. Hence the results are more reliable if the procedure is done within a short span of time.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in K.S.R Institute of Dental Science and Research, Tiruchengode, where the extracted teeth were collected from patients who underwent dental extraction procedure. Hundred clinically sound human permanent teeth were collected randomly along with the respective blood samples from patients with age ranging 11-70 who reported for extraction due to orthodontic treatment procedure and poor periodontal status. The study was divided into two groups: Group I (freshly extracted teeth) comprised of 50 teeth, which were analyzed within a period of a week. Group II (teeth kept for long period of time in soil) comprised of 50 teeth that were collected and kept for a period of 4-6 months and then analyzed. The method used to determine the blood group from teeth was modified absorption elution technique. In Group I (n = 50), when pulp was compared with the reference, 46 teeth showed positive results and the sensitivity of pulp in comparison to blood was found to be 92%. Dentin when compared with blood, 41 teeth showed positive results and the sensitivity was 82% .Teeth (pulp +dentin) correlated in 39 samples with a sensitivity of 78%. In Group II (n = 30), for pulp, 39 samples showed positive results and eleven showed negative result with a sensitivity of 78%. Comparison of dentin with blood showed 21 samples correlating reference group with a sensitivity of 42% .Complete correlation for teeth was seen in only 21 samples with 29 negative results showing a sensitivity of 42%. In this study, we conclude that if teeth are the only source of identification of a person in forensics, pulp tissue is more significant. Though dentin also possesses blood group antigens, results may not reliable. Though there are many methods to determine the blood group antigens in teeth, this method is less expensive and does not require sophisticated equipments.

LIMITATIONS OF THE STUDY

1. In this study the various parameters like age, sex, teeth taken for the analysis and blood groups were compared within the group since the sample size was less. Inter group analysis of each parameter can be done if the samples are equally distributed in each category. This can be done when large samples are analyzed.

2. The storage medium taken here was soil. Other medium of storage can also be analyzed like sea water where there will be change in pH which may influence the results.

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ANNEXURE

ANNEXURE – I

INFORMED CONSENT

I _____ hereby declare that I clearly understood the procedures of the study. Also, I declare that I give permission for the above mentioned individual/organization/hospital to do the procedure to the individual/organization listed above.

Signature_____ Date_____

I have explained the above and answered all questions asked by the participant.

Signature of Investigator_____ Date_____

I

ஒப்புகை வாக்குமூலம்

..... ஆகிய நான் மேற்கூறிய ஆராய்ச்சி படிப்பின் வழிமுறைகளைத் தெளிவாகப் புரிந்து கொண்டேன். மேலும் நான் இந்த ஆராய்ச்சிப் படிப்புக்கான வழிமுறைகளை மேற்கொள்வதற்கும், அதன் பரிசோதனை முடிவுகளை தெரிந்து கொள்ளவும் முழுமையாக அனுமதிக்கிறேன்.

.....

நோயாளியின் கையொப்பம் தேதி.....

நான் மேற்கூறிய ஆராய்ச்சிப் படிப்பிற்கான விதிமுறைகள் மற்றும் அது குறித்த நோயாளியின் சந்தேகங்களையும் தெளிவாக விளக்கியுள்ளேன்.

.....

மருத்துவரின் கையொப்பம் தேதி.....

ANNEXURE – II

INSTITUTIONAL ETHICAL COMMITTEE

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Member Secretary
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Principal,
KSR Institute of Dental Science & Research,
KSR Kalvi Nagar, Tiruchengode.

Ref.: 052 /KSRIDSR/EC/2014 **Date : 09.01.2014**

To

Dr.A.Nilophar,
Postgraduate Student,
Dept. of Oral Medicine & Radiology,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "ABO BLOOD GROUPING FROM HARD AND SOFT TISSUES OF TEETH: BY MODIFIED ABSORPTION – ELUTION TECHNIQUE" presented before the ethical committee on 7th Jan. 2014 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary,
(Dr.G.S.Kumar)