INFLUENCE OF RBC COUNT AND HAEMOGLOBIN
CONCENTRATION ON PLATELET RICH FIBRIN NETWORK AMOMG DIFFERENT AGE GROUPS AND GENDER- A CROSS SECTIONAL STUDY.

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ABSTRACT

AIM: To determine the influence of RBC count and hemoglobin concentration on the density of PRF network among individualsof different age groups and gender.

MATERIALS AND METHODS: 82 individuals were dividedinto three groups based on their age, Group 1: 20-30 years, Group 2: 30-45 years, & Group 3: 45-60 years. 3-4 ml of venousblood samples were collected, PRF were prepared and it was subjected to histological analysis to determine the age and genderrelated changes in the density of PRF network. Hematological parameters such as Hb concentration and RBC count were also evaluated and correlated with the density of PRF network.

RESULTS: Group 1 showed predominantly dense fibrin network, Group 2 showed mixed pattern including both dense and loose fibrin network and Group 3 showed predominantly loose fibrin network. However the difference in fibrin network among different age groups was not statistically significant (p value-0.14). Among the gender, males showed dense fibrin network compared to females which was statistically significantwith p value-0.03. There was no statistically significant correlation between RBC count and density of fibrin network with p value-0.11. There was a statistically significant correlation between Hb level and density of fibrin network with p value-0.05.

CONCLUSION: The PRF which is widely used in periodontal regenerative therapy differ in densities between male (dense network) and female (loose network).It is found to be denser in individuals with high hemoglobin concentration.

Key words: Platelet Rich fibrin, RBC, HB, Densenetwork, loose network.

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease which leads to the destruction of both soft and hard tissues of the periodontium. The ultimate goal of periodontal therapy is to restore and regenerate the lost periodontal structures and its functions.[1] Wound healing is a complex biological process which include different cell interactions and growth factors to re-establish the lost periodontal tissues.[2] In periodontal regeneration healing occurs with the formation of new periodontal ligament, cementum and alveolar bone.[1] Different regenerative modalities are available in periodontal regenerative therapy which include root conditioning agents, enamel matrix derivative, bone grafts, GTR, platelet concentrates, stem cells, gene therapy etc.[2]

Platelet concentrates are considered to be rich in growth in factors and it is widely used in regenerative dentistry. Fibrin glue which was described in 1970 was initially used in periodontal regenerative therapy.

Later Marx et al in 1998 identified platelet rich plasma (PRP) which is an autologous modification of fibrin glue.[3] It has a very rigid network which is not favorable for the cytokine fixation and cellular migration. Later, Choukroun et al in 2001 identified second generation platelet concentrate, termed platelet rich fibrin (PRF).[4] It has a flexible fibrin matrix network favorable for cytokine entrapment and cellular migration. The formation of fibrin blood clot is considered to be the main step in the process of wound healing. The normal blood clot contain 95% of RBC, 5% of platelets and 1% of white blood cells, whereas PRF blood clot contain more than 95% of platelets and thereby it speeds up the wound healing process.

The density of the PRF fibrin matrix obtained after centrifugation may be related to the age, gender, genetic factors, hematological parameters (RBC count, Hb concentration) etc.[5] Thus density of fibrin network plays a major role in predicting the success of periodontal regeneration. Many studies have been done so far regarding the influence of age and gender on the density of PRF fibrin network. Very few literature evidence is available regarding the influence of hematological parameters on density of PRF network. Hence this study was conducted in order to determine the influence of RBC counts and hemoglobin concentration on the density of platelet rich fibrin network among different age groups and gender.

MATERIALS AND METHODS

The study was conducted in the Department of Periodontics, K. S. R Institute of Dental Science and Research from June 2019 - October 2019. Patients who reported to the department, were educated about the significance of this study and those who showed willingness for the study were recruited and written informed consent were obtained from them. Finally eighty two subjects were involved in this study according to the following inclusion and exclusion criteria,

Inclusion criteria:

Age = 20-60 years

Both gender

Systemically healthy individuals

Exclusion criteria:

Smoking

Pregnant and lactating women

Hematological disorders

Medications- antiplatelet andanticoagulants

The eighty two subjects (37-Males, 45- Females) were divided into three groups based on age as follows,

Group 1 (20-30 Years): n=29 Group 2 (30-45 Years): n=27 Group 3 (45-60 Years): n=26

HEMATOLOGICAL ANALYSIS

The patients were subjected to hematological analysis to determine RBC count and hemoglobin concentration in the laboratory of K.S.R institute of dental science and research.

PLATELET RICH FIBRIN PREPARATION

3-4 ml of intravenous blood sample (antecubital vein) was collected from the individuals using a 5ml disposable plastic syringe. Once blood was collected, it was transferred immediately to a plain glass tubes withoutany anticoagulant. The blood samples were centrifuged
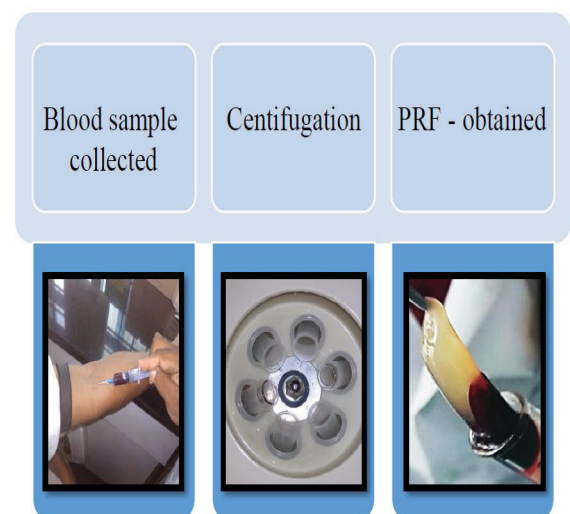

Fig 1: Steps in PRF preparation STEPS IN TISSUE PREPARATION (PRF) FOR HISTOLOGICAL ANALYSIS

Fixing: Obtained PRF clots from each individual were placed in a perforated stainless steel cassette and subjected to fixing in a formalin solution for 24 hours.

Tissue processing: After fixation, the PRF clots in a stainless steel cassette were passed through the sequence of various tissue processing solutions such as 10% formalin, 60%, 70%, 80%, 90%, and 100% isopropanol alcohol, xylene (two changes).

Embedding: After tissue processing, the PRF clots were embedded in a paraffin wax in a wax mould (Leuchar blocks).

Sectioning: Individual wax blocks were sectioned into slices using a Leica microtome in 4μm thickness.

Dewaxing: It was done by heating the slides at 550C and later it was immersed into xylene to eliminate wax.

Tissue staining: Slides were stained using hematoxylin and eosin stain. Slides were numbered and subjected to histological slide analysis by using compound microscope at 10x, 20x, 40x, 100x magnification in the Department of Oral Pathology, K.S.R institute of dental science and research.
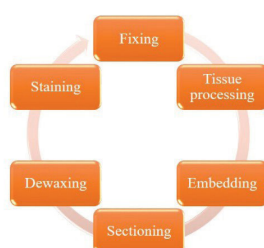

Fig.2: Steps in tissue preparation for histological analysis

Fig 2a: Fixing   Fig 2b: Processing Fig 2c: Sectioning   Fig 2d: Prepared slides

STATISTICAL ANAYSIS

The results were presented in a numerical form in MS excel, computed, analyzed using statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, Version 20.0 for Windows). The test of association was done using chi square test with p value set at 0.05 significance level.
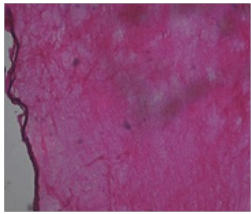
RESULTS

The stained slides were evaluated under compound microscope at 10x, 20x, 40x, 100x magnification for the type of fibrin network (dense, mixed and loose fibrin network). The slides showed RBCs, fibrin network with entrapment of platelets and WBCs. The density of fibrin network were evaluated according to the age groups and gender, RBC count and hemoglobin concentration.
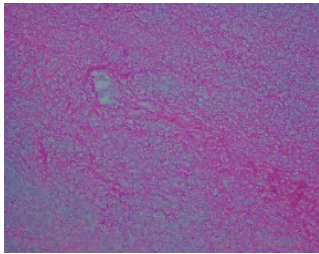
DENSITY OF FIBRIN NETWORK AND AGE

Among the three groups involved in this study, Group 1 subjects (20-30 years) showed increased arrangement of homogenous dense fibrin network than the loose fibrin network. Group 2 subjects (30-45 years) showed mixed network (i.e) it showed almost equal distribution of both dense and loose fibrin network. Group 3 subjects (45-60 years) showed increased distribution of loose fibrin network than dense fibrin network. However the difference in the densities of fibrin network among the groups with respect to age was not statistically significant with p value -0.14.
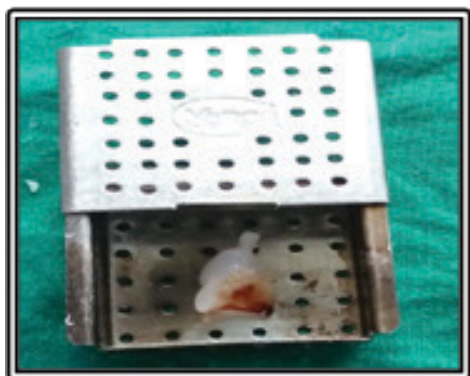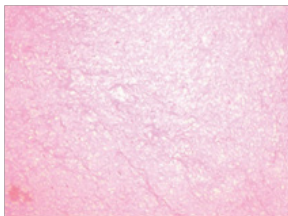
Group:3 (Loose network)


Group:2 (Mixed network)

Group:3 (Loose network)

Fig 3: Histological slides showing the density of fibrin network at 10x in different age groups

From the results it could be considered, that the age group of 30-45 years can be considered as a transition stage where the pattern of fibrin network showed both dense and loose network. And also, when the age advances more than 60 years, PRF clot would show predominantly a loose fibrin network with weak entrapment of platelets and WBCs.
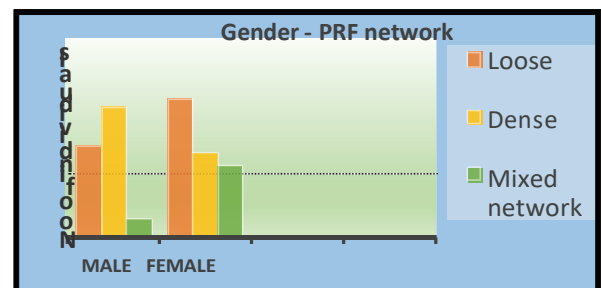
DENSITY OF FIBRIN NETWORK AND GENDER:

Among the gender, males showed predominantly dense fibrin network and females showed predominantly loose fibrin network which was statistically significant with p value-0.03.
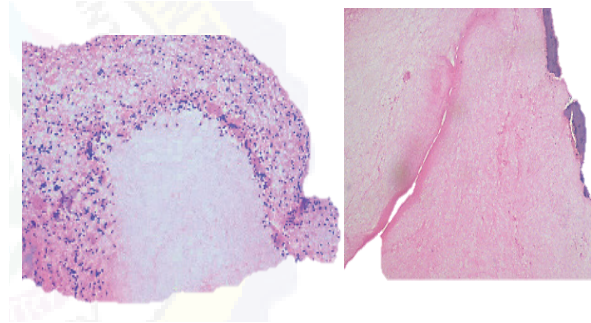
Male (dense network) Female (loose network)

Fig 4: Histological slides showing the pattern offibrin network at 10x in different gender

Graph 1: Relationship between PRF network and gender

INFLUENCE OF HAEMATOLOGICAL PARAMETERS ONDENSITYOF FIBRIN NETWORK:

RBC count and hemoglobin concentrationdetermined for each individual were compared with the density of fibrin network. Since RBC count and Hb concentration were quantitative data, it couldnot be compared with the density of fibrin network (qualitative data). Hence the individuals were categorized into three groups based on their RBC count and Hb concentration (highest and lowest limit) as follows,

RBC COUNT

2.5-3.5 million cells / cu mm - showedpredominantly loose fibrin network.

3.5-4.5 million cells / cu mm - showed mixed network.

4.5-5.5 million cells / cu mm - showedpredominantly dense fibrin network.

The variation in density of fibrin network among the

groups with respect to RBC count was found to be statistically not significant with p value – 0.11.

Hb concentration:

6-9gm% - showed predominantly loose fibrin network.

9-12gm% - showed mixed network.

12-15% - showed predominantly dense fibrin network.

The variation in the density of fibrin network among the groups in respect to Hb concentration was found to be statistically significant with p value – 0.05.

Even though the density of fibrin network can be predicted as dense and loose network in histologic analysis, there is no specific index or criteria to denote the fibrin strands as loose and dense.
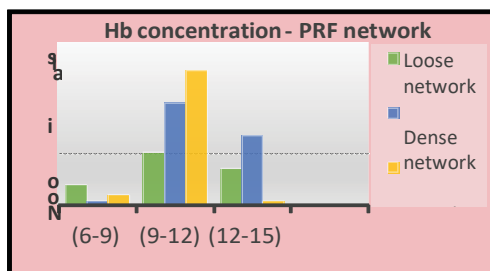
DISCUSSION

Dohan et al in 2009 classified the platelet concentrates into 1. Pure Platelet-Rich Plasma (P-PRP) – or Leukocyte-Poor Platelet-Rich Plasma, 2. Leukocyte- and Platelet-Rich Plasma

(L-PRP), 3. Pure Platelet-Rich Fibrin (P-PRF) – or Leukocyte Poor Platelet-Rich Fibrin, 4. Leukocyte- and Platelet-Rich Fibrin (L-PRF) products.[6] PRF which is a second generation platelet concentrate has many advantages compared to first generation platelet concentrate, platelet rich plasma. The advantages includes, easy preparation process with one spin centrifugation step, shows flexible network with slow release of growth factors for a period of 7-11 days.

Laurens et al in 2006[7] stated that the density, porosity and permeability of fibrin network plays a major role in wound healing process. However the network of PRF show alterations according to the age, gender,

systemic condition of the individuals. These factors in turn would determine the success of periodontal regeneration.

The results of the present study revealed that in individuals with more than 45 years of age, the density of PRF network become loose with weak entrapment of WBCs and platelets.
Triveni kala[8] et al 2017, analysed the PRF network pattern changes in different age groups and gender and found a decrease in the density of network as age advances. Yajamanya[6] et al 2016 also conducted a similar study and found a noticeable decrease in the density of network in older age groups. Even though the results of the above mentioned studies correlates with the present study, the grouping of individuals according to the age criteria differs in this study which could be considered as a limitation of this study.

Comparing the gender and PRF network, males showed p r e d o m i n a n t l y dense fibrin network than the females. Graph 2: Relationship between PRF network and Hb concentration

This observation is similar with the studies conducted by Yajamanya et al and and Triveni kala et al.

There was no scientific evidence in the literature regarding the influence of hematological parameters on the density of PRF network. Miron et al in 2018[9] conducted a study to determine the effect of age and gender on the size of PRF membrane. They found a larger PRF membrane in females and older age group individuals compared to males and younger individuals. They stated that this difference may be due to the alterations in hematocrit value.

Hence this study was conducted to evaluate the relationship between the hematological parameters (RBC and Hb) and density of network. However, in this study RBC concentration failed to show the statistically significant relationship. Whereas individuals with high Hb concentration showed predominantly dense fibrin network which was statistically significant. From this study, it is concluded that hemoglobin concentration can also be considered as a factor in determining the density of PRF network.

From this study it is concluded that age, gender and hematological parameters has a strong influence in predicting the success of periodontal regeneration.

But there is no scientific evidence in the literature regarding the histological evaluation of periodontal regeneration with respect to different densities of PRF network. Thus in future many histological studies has to be conducted in order to strongly substantiate the role of these factors on the success of periodontal regeneration. Moreover, after the identification of T-PRF (Tunali et al -2014)[10] which is a third generation platelet concentrate many studies are focusing on its efficacy on periodontal regeneration. It has more dense fibrin network with slow resorption rate compared to PRF. Future studies are needed to compare the influence of age, gender and hematological parameters on the density T-PRF network as well.

CONCLUSION

PRF which is widely used in periodontal regenerative therapy is known to be affected by factors such as age, gender and hematological parameters. Thus these factors have to be monitored while planning for successful periodontal regenerative therapy.

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