2017

Effect of Platelet Rich Plasma on Osseointegration of Dental Implant

Hossain, Md. Amzad

University of Rajshahi

EFFECT OF PLATELET RICH PLASMA ON OSSEOINTEGRATION OF DENTAL IMPLANT

THESIS SUBMITTED FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

IN THE

INSTITUTE OF BIOLOGICAL SCIENCES

UNIVERSITY OF RAJSHAHI, BANGLADESH

By

Md. Amzad Hossain

BDS, MSc, MS, FCPS

JUNE 2017

INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI
RAJSHAHI, BANGLADESH

DEDICATED

TO

MY BELOVED PARENTS

DECLARATION

I do hereby humbly declare that the whole research work submitted as a thesis entitled "EFFECT OF PLATELET RICH PLASMA ON OSSEOINTEGRATION OF DENTAL IMPLANT" in the Institute of Biological Sciences, University of Rajshahi, Bangladesh for the degree of Doctor of Philosophy is the result of my own investigation and observation.

I further declare that the thesis or part of it has not been submitted elsewhere for any degree or diploma.

15.06.2017

Md. Amzad Hossain

(Candidate)

CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF PLATELET RICH PLASMA ON OSSEOINTEGRATION OF DENTAL IMPLANT"** submitted in the Institute of Biological Sciences, University of Rajshahi for the degree of Doctor of Philosophy is a bonafide research work carried out by Md. Amzad Hossain under our supervision. The results of the investigation embodied in the thesis are original and the thesis or part of it has not been submitted elsewhere for any degree or diploma.

We are forwarding the thesis for examination and evaluation.

19/06/2017

Dr. K. A. M. Shahadat Hossain Mondal
Professor
Institute of Biological Sciences
University of Rajshahi
Rajshahi, Bangladesh

(Supervisor)

15/06/2017

Dr. Motiur Rahman Molla
Professor
Dept. of Oral & Maxillofacial Surgery
Anwer Khan Modern Medical College
Dhaka, Bangladesh

(Co-Supervisor)

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and indebtedness to my supervisors Dr. K. A. M. Shahadat Hossain Mondal, Professor, Institute of Biological Sciences, University of Rajshahi, Bangladesh and Dr. Motiur Rahman Molla, Professor, Department of Oral & Maxillofacia Surgery, Anwer Khan Modern Medica College, Dhaka, Bangladesh for their guidance and valuable advice throughout the research work.

I would also like to express my gratitude to Late Professor Dr. M. A. Mannan, Department of Zoology, University of Rajshahi, Bangladesh for his kind help and advice during the early phase of the research programme.

I am grateful to the Director, Institute of Biological Sciences, University of Rajshahi for providing all necessary facilities to conduct my research work.

Finally, I am indebted to all my patients and their attendants.

Md. Amzad Hossain

ABSTRACT

Title: Effect of platelet rich plasma on osseointegration of dental implant.

Background: Dental Implant is an alloplastic material that serves as root analogue for missing tooth. This is surgically inserted into the soft and hard tissue of the jaws primarily as prosthodontic foundation. Implant therapy offers many advantages over conventional fixed or removable treatment options and in many cases is the treatment of choice. The clinical success of implant therapy in edentulous and partially edentulous patients is well documented and clinicians realize the benefits of adopting implant therapy in their practices. Presently it is an integral part of mainstream dentistry, and a highly predictable and unique treatment modality to replace missing teeth. The long-term clinical success of dental implant is related to its early and optimal osseointegration. Despite the ongoing improvement in implant characteristics, bone intrinsic potential for osseointegration may be stimulated with adjuvant therapies to standard surgical procedures to achieve the best possible implant osseointegration into the adjacent bone and to ensure its long term success. For this purpose, various pharmacological, biological or biophysical modalities have been developed, such as bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins. In the present study, autologous platelet rich plasma (PRP) was used as an adjuvant therapy to standard surgical procedure of dental implant. The PRP reduces of post operative complications, stimulates wound healing and enhances bone regeneration around dental implant providing the most advantageous environment for its acceptance. It is safe due to its autologous nature and free from risk of

cross reactivity, immune reaction or disease transmission, and it can be produced as needed from patient's own blood.

Objective: To evaluate the effect of platelet rich plasma on osseointegration of dental implant.

Materials and Methods: This interventional prospective controlled clinical trial was carried out in Face-bow Institute of Implant Dentistry & Prosthodontics, Mirpur, Dhaka; and Anwer Khan Modern Medical College & Hospital, Dhanmondi, Dhaka with the affiliation of Institute of Biological Sciences (IBSc) of University of Rajshahi from August 2012 to December 2016. In this study, a total 300 patients with single missing tooth were consecutively selected by thorough medical and dental history as well as meticulous clinical examination, and radiological and biochemical investigations supporting the specific exclusion and inclusion criteria. Of the 300 patients, 150 were treated with dental implants using platelet rich plasma (PRP) as an adjuvant therapy (Study Group) and other 150 were treated with dental implants conventionally without using any adjuvant therapy (Control Group). Till the final evaluation phase 4 patients were dropped and 2 patients were excluded from study group, and 5 patients were dropped and 1 patient was excluded from the control group. Ultimately, a total of 288 patients, 144 from each group were evaluated in this study. Standard pre-surgical sterilization protocol was maintained for every patient and an ethical standard procedure was followed for surgical placement of every implant into selected site. All patients were previously informed about implant system, its merits and demits, and possible alternative treatments. Every patient gave the written informed consent before surgery. Under prophylaxis antibiotic and local anaesthesia horizontal off crestal with

required vertical releasing incisions were given and full thickness subperiosteal flap was reflected. Surgical stents having guide channel was used to place the implant in correct position and angulation. Osteotomy was done using the sequential diameter of drills attached in a reduction gear hand piece along with a physio-dispenser having internal and external irrigation system to prevent excessive heat generation. The speed of drill was ranged between 1200 and 1500 rpm with copious irrigation. In the mean time PRP was prepared from the patient's own blood drawn before starting the surgery by a two phase spinning system using table top centrifugal machine. Implant was placed maintaining all asepsis measures with meticulous precautions. The prepared PRP was placed at the surgical site all around the implant. Suturing was done and the instructions were given for maintenance of the implant as well as his/her oral health. Patients were advised to come for recall visits after 1^{st}, 4^{th}, 8^{th}, 12^{th}, and 16^{th} week for evaluation of implant sites. Each patient was monitored intensively to assess the pain and swelling for each day of first week. Data were collected by history, clinical and radiographic investigations on outcome variables of pain, swelling, bone resorption, imagistic value and stability at follow up visits. Collected data were edited and calculated for presentation as results. Unpaired 't' test was done and P value < 0.05 was considered the result as statistically significant.

Results: Results showed that a total 288 implant sites of 288 patients were evaluated in this study. Of them 152 (52.78%) patients were males and 136 (47.22%) were females. The ages of the patients ranged between 22 and 82 years with the mean age 46.64 (±13.78) years. Out of the 288 implants, 144 (50%) were placed with PRP and cosidered as study group,

and other 144 (50%) were placed without platelet rich plasma and considered as control group.

Postoperative discomfort of the patients were evaluated in terms of pain and swelling. Postoperative pain was evaluated by a visual analogue scale (VAS). All patients reported mild pain on the first day of surgery and the mean VAS score was 22.67±14.19 for study group and that was 25±13.42 for the control group. The mean VAS scores were 15.72±13.92, 6.00±7.95, 3.13±5.41, 1.25±2.61 and 0.42±1.31 for study group on the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, and 6^{th} day of surgery respectively; and the values for control group were 18.46±12.87, 9.4±10.85, 5.42±8.58, 2.86±4.53, 1.75±3.93 and 0.58±2.09 on the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day of surgery respectively.

Postoperative swelling was evaluated by verbal rating scale (VRS). The VRS scores were 2.31±1.05, 2.26±0.90, 2.28±0.90, 2.14±0.82, 1.51±0.63, 1.31±0.46 and 1.14±0.35 for study group on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day of surgery respectively. The values for the control group were 2.51±1.04, 2.85±0.94, 2.82±1.11, 2.64±1.10, 2.32±1.13, 1.99±1.15, 1.58±0.72 and 1.17±0.37 on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} day of surgery respectively. No patient of study group reported swelling after 7^{th} day, but a few patients of control group reported pain till 8^{th} postoperative day.

The mean imagistic values were rated on 1^{st}, 4^{th}, 8^{th}, 12^{th} and 16^{th} week of implant placement. In the first week, the baseline mean imagistic value was -3±0.00 in both groups of implants. The mean imagistic values of study group reached -1.76±0.68 at 4^{th} week, 0.60±1.84 at 8^{th} week, 3.83±0.99 at 12^{th} week and 4.94±0.95 at16^{th} week. On the other hand, the

values of control group were -2.28±0.81, -1±1.75, 1.75±1.28 and 2.65±0.61 at 4th week, 8th week, 12 week and 16th week respectably.

The mean marginal (vertical) bone loss was assessed at baseline and 2nd surgery. For the study group, the mean vertical bone height was 2.08±0.70 mm at baseline and 1.37±0.64 mm at 2nd surgery, and the mean bone loss was 0.71±0.25 mm. For the control group, the values were 2.07±0.70 mm and 0.86±0.35 mm at baseline and 2nd surgery respectively, and the mean vertical bone loss was 1.21±0.43 mm.

The bucco-lingual (horizontal) bone loss at the sites of implants was measured. The mean bucco-lingual bone width for study group was 6.95±1.15 mm and that was 6.93±1.14 for control group at the time of implant placement. The mean width was 9.19±0.96 mm for the study group at the time of 2nd surgery and mean bucco-lingual bone loss was 0.76±0.43 mm. On the other hand, the mean bucco-lingual bone width was 5.85±0.80 mm at time of 2nd surgery and the bone loss was 1.08±0.62 mm.

Stability of each implant was assessed at the time of placement and at the time of second surgery when abutment was attached. Periotest and implant stability quotient devices were used to assess primary and secondary stability for each implant. No clinically naked eye mobility was found in any implant at primary and secondary stage of assessment.

The baseline periotest value at 1st surgery was 10.33±1.06 for study group and that was 10.29±1.05 for control group. The value became -5.29±1.10 for study group and that was -3.90±1.33 for control group after 16th week

at the time of second surgery. The value changed from 1st surgery to 2nd surgery was 15.63±0.49 for study group and that changed value was 14.19±0.52 for control group. Negative value indicates stability and if greater the negative value, greater will be the stability of implants.

The baseline ISQ at 1st surgery was 33.47±2.45 for study group and that was 33.43±2.04 for control group. After 16th week at the time of 2nd surgery ISQ was 81.74±1.22 for study group and that was 61.39±1.24 for control group. The difference of ISQ from 1st surgery to 2nd surgery was 48.26±1.22 for study group and that was 27.96±0.80 for control group.

In addition to hypothesis testing and beyond the objective of the study, periimplant indices of 180 implants were assessed after one year of prosthetic loading. At the baseline the mean plaque index was 0.00 ± 0.00 for both the study and control groups. After one year of prosthetic loading the value became 0.56±0.50 for study group and that became 1.11±0.74 for control group. Similarly, at the baseline the mean bleeding on probing was 0.00 ± 0.00 for both study and control groups. After one year the value was 0.33 ± 0.47 for study group and that was 1.00 ± 0.82 for control group. The baseline periimplant probing depth was 1.56 ± 0.50 mm for study group and that was 1.44 ± 0.50 mm for control group. After one year of loading the values were 2.06 ± 0.50 mm and 2.44 ± 0.55 mm for study group and control group respectively. The differences of indices from baseline to one year were statistically highly significant.

Conclusion: Based on the results of this study it can be concluded that platelet rich plasma enhances osseointegration of dental implant.

CONTENTS

		Page No.
	Acknowledgements	v
	Abstract	vi
	List of Tables	xviii
	List of Figures	xix
	List of Abbreviation	xx
CHAPTER 1	**INTRODUCTION**	1-13
1.1.	Introduction	2
1.2.	Rationale	10
1.3.	Hypothesis	12
1.4.	Objectives	13
CHAPTER 2	**REVIEW OF LITERATURE**	14 - 41
2.1.	Human tooth and its loss	15
2.2.	Replacement of lost tooth	19
2.3.	Implant dentistry	20
2.3.1.	Definition of dental implant	20
2.3.2.	History and Concept	21
2.4.	Osseointegration	23
2.4.1.	Definition of osseointegration	23
2.4.2.	History of osseointegration	24
2.4.3.	Biology of osseointegration	25
2.4.4.	Factors enhancing osseointegration	28
2.4.4.1.	Implant related factors	28

2.4.4.2.	The host bone status and its healing potential of bone	30
2.4.4.3.	Mechanical stability	30
2.4.4.4.	Use of adjuvant therapies	30
2.5.	Platelet rich plasma	31
2.5.1.	Definition and composition	31
2.5.2.	Mechanism of action of PRP	32
2.5.3.	Application of platelet rich plasma	33
2.5.3.1.	Application in regenerative activity	34
2.5.3.1.1.	On tooth supporting structures	34
2.5.3.1.2.	On sequence of bone regeneration	34
2.5.4.	Rationale to use PRP rather than other adjuvant therapy	35
2.6.	Procurement of platelet rich plasma	37
2.6.1.	Systems for procurement	37
2.6.1.1.	One touch automated PRP systems	37
2.6.1.2.	Plasmapheresis	37
2.6.1.3.	Manually PRP preparation	37
2.6.2.	General production and activation of PRP	38
2.6.3.	A simple chair side technique for PRP preparation	49
2.7.	Use of PRP in implant surgery	40
CHAPTER 3	**MATERIALS AND METHODS**	42 - 70
3.1.	Design of study	43
3.2.	Place of study	43
3.3.	Duration of study	43
3.4.	Study population	43
3.5.	Sampling technique	43

3.6.	Sample size	43
3.7.	Sample size determination	44
3.8.	Ethical consideration	45
3.9.	Patient selection	45
3.9.1.	Inclusion criteria	46
3.9.2.	Exclusion criteria	47
3.10.	Clinical examination	48
3.11.	Diagnostic cast analysis	48
3.12.	Diagnostic wax up	49
3.13.	Investigations advised for each patient	49
3.14.	Diagnosis	49
3.15.	Treatment plan	50
3.16.	Implant system and size selection	50
3.17.	Sterilization protocol	51
3.17.1.	Instruments preparation	52
3.17.2.	Unit preparation	52
3.17.3.	Room preparation	52
3.17.4.	Operator and assistant	52
3.18.	Pre-surgical protocol	53
3.19.	Surgical protocol	54
3.20.	PRP preparation and application	55
3.21.	Implant installation	56
3.22.	Postoperative care	57
3.22.1.	Post operative instructions	57
3.22.2.	Post operative follow up	59
3.23.	Evaluation of osseointegration	59
3.23.1.	Clinical evaluation	59
3.23.2.	Pain and swelling in terms of discomfort	60

3.23.3.	Imagistic evaluation by radiograph	61
3.23.4.	Evaluation of vertical bone height by perioprobe	61
3.23.5.	Evaluation of vertical bone height by X-ray tracing	62
3.23.6.	Evaluation of horizontal bone width by cast analysis and perioprobe	63
3.23.7.	Evaluation of implant stability	64
3.23.7.1.	Assessment of stability with periotest	64
3.23.7.2.	Assessment of stability with implant stability quotient	66
3.23.8.	Assessment of periimplant tissue after loading	66
3.23.9.	Data collection	68
3.23.10.	Statistical analysis	69
3.23.11.	Whole method	69
CHAPTER 4	**RESULTS**	71 - 85
4.1.	Distribution patients based on sex	72
4.2.	Distribution of patients based on age	73
4.3.	Distribution of implants based study and control group	74
4.4.	Evaluation of postoperative pain of patients	75
4.5.	Evaluation of postoperative swelling of patients	77
4.6.	Evaluation of imagistic values of implants	79
4.7.	Evaluation of vertical bone height around Implants	80
4.8.	Evaluation of horizontal bone loss around Implants	81
4.9.	Evaluation of implant stability by periotest	82

4.10.	Evaluation of implant stability quotient	83
4.11.	Evaluation of periimplant indices after prosthetic loading	84
CHAPTER 5	**DISCUSSION**	86 -110
CHAPTER 6	**CONCLUSION AND RECOMMENDATION**	111 - 113
6.1.	Conclusion	112
6.2.	Recommendation	112
6.3.	Limitation of the study	113
CHAPTER 7	**SUMMARY**	114-121
	REFERENCES	122 - 150
	APPENDICES	151- 180
Appendix 1	History sheet	152
Appendix 2	Informed consent for dental implant placement	156
Appendix 3	Data collection sheet	160
Appendix 4	Certificate of ethical clearance	163
Appendix 5	Mean age patients based on sex	164
Appendix 5a	Distribution of frequency and percentage of patients based on sex	164
Appendix 6	Distribution patients based on age	165
Appendix 7	Evaluation of postoperative pain of the patients	166
Appendix 7a	Visual analogue scale for postoperative pain	166

Appendix 8	Evaluation of postoperative swelling of the patients	167
Appendix 8a	Verbal rating scale for postoperative swelling	167
Appendix 9	Evaluation of imagistic values of implants	168
Appendix 10	Evaluation of vertical bone height around implants	169
Appendix 10a	Method of vertical bone measurement by X-ray tracing	169
Appendix 11	Evaluation of horizontal bone around implants	170
Appendix 11a	Cast analysis to measure horizontal bone	170
Appendix 12	Evaluation of implant stability by periotest values	171
Appendix 12a	Periotest values used in the study	171
Appendix 13	Evaluation of implant stability quotient	172
Appendix 14	Evaluation of periimplant indices	173
Appendix 14a	Periimplant indices used in the study	174
Appendix 15	Measurement of peiimplant probing depth	175
Appendix 16	Comparative anatomy of tooth and implant	176
Appendix 17	An implant fixture	177
Appendix 18	Histological feature of osseointegration	178
Appendix 19	Steps of PRP preparation	179
Appendix 20	Steps for surgical placement of implant	180

LIST OF TABLES

Table No.	Title	Page No.
Table 1	Some selected references on the RCTs using PRP	41
Table 2	Evaluation of postoperative pain of the patients	76
Table 3	Evaluation of postoperative swelling of the patients	78
Table 4	Evaluation of imagistic values of implants	79
Table 5	Evaluation of vertical bone height around implants	80
Table 6	Evaluation of horizontal bone loss around implants	81
Table 7	Evaluation of implant stability by periotest values	82
Table 8	Evaluation of implant stability quotient	83
Table 9	Evaluation of periimplant indices after 1 year of prosthetic loading	85

LIST OF FIGURES

Figure No.	Title	Page No.
Figure 1	Dental Implant	51
Figure 2	Flowchart of the study	70
Figure 3	Distribution of patients based on sex	72
Figure 4	Distribution of patients based on age	73
Figure 5	Distribution of implants based on Study and control group	74

LIST OF ABBREVIATIONS

Abbreviation	Elaboration
AD	Anno Domini
APG	Autologus Platelet Gel
BC	Before Christ
BOP	Bleeding on probing
cc	Cubic centimeter
CE	*Confomité Européenne* (European Conformity)
CPDS	Citrate Phosphate Dextrose Solution
ECGF	Epithelial cell growth factor
EGF	Epidermal Growth Factor
EGF	Epithelial growth factor
FAD	Food and Drug Administration
Ff	Fibrinogen
Fn	Fibronectin
FPDs	Fixed partial dentures
GF	Growth factor
gm	gram
Hg	Mercury
HIV	Human Immunodeficiency Virus
IGF	Insulin like growth factor
IL	Interleukin
ISO	International Organization of Standardization
ISQ	Implant stability quotient
Lab	Laboratory
MBL	Marginal bone loss
ml	milliliter
MRSA	Methicillin resistant *Staphylococcus aureus*_

Ncm	Neuton centimeter
Oc	Osteocalcin
On	Osteonectin
PDAF	Plaelet Derived Angiogenesis Factor
PDEGF	Platelet Derived Endothelial Growth Factor
PDGF	Platelet Derived Growth Factor
PEP	Platelet Enriched Plasma
PF	Platelet Factor
PI	Plaque index
PPD	Probing pocket depth
PPP	Platelet poor plasma
PR	Platelet Releasate
PRC	Platelet Rich Concentrate
PRP	Platelet Rich Plasma
PT	Periotest
PTV	Periotest value
RBC	Red Blood Cell
RFA	Resonance frequency analysis
rpm	Rotation per minute
TGF	Transforming Growth Factor
Ti	Pure Titanium
TMD	Temoporomandibular disorder
Tsp	Thrombospondin
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
Vn	Vitronectin
VRS	Verbal Rating Scale

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. Introduction

Dental Implant is an alloplastic material that serves as root analogue for missing tooth. This is surgically inserted into the soft and hard tissue of the jaws primarily as prosthodontic foundation. It thus provides support and retention of dental prosthesis (Misch 2011). Compared to all other dental disciplines, implant dentistry has enjoyed far more innovation and progressive development in recent years. Implant therapy offers many advantages over conventional fixed or removable treatment options and in many cases is the treatment of choice (Jivraj and Chee 2006). The clinical success of implant therapy in edentulous and partially edentulous patients is well documented and clinicians realize the benefits of adopting implant therapy in their practices. Presently, it is an integral part of mainstream dentistry, and a highly predictable and unique treatment modality to replace missing teeth (Adell *et al.* 1990, Lindh *et al.*1998).

The biological fixation between the dental implant and jaw bones is considered a prerequisite for the long-term success of implant supported prostheses (Arthur *et al.* 2010) which is related to its early osseointegration (Lavenus *et al.* 2010).

Osseointegration is defined as "a time dependent healing process whereby clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading" (Zarb and Albrektsson 1991). Osseointegration refers to a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant. Osseointegrated implant provides a foundation to support prosthesis and has the ability to transmit occlusal forces directly to bone. In this phenomenon the implant must be made of an inert

biocompatible material to be in direct contact with bone; without soft tissue, scar tissue, cartilage or ligament interface. Currently, an implant is considered as osseointegrated when there is no progressive relative movement between the implant and the bone with which it has direct contact. When Osseointegration occurs, the implant is tightly held in place by the bone developing a close bond between the two. In general, it is the wound healing with bone regeneration around dental implant after its surgical placement. The process typically takes four to six months to occur (Albrektsson *et al.* 1981, Branemark 1983, Carlsson *et al.* 1986, Mavrogenis *et al.* 2009, Nandal *et al.* 2014).

The concept of osseointegration was first developed and the term was coined by orthopaedic surgeon Dr. Per-Ingver Branemark, Professor at The Institute for Applied Biotechnology, University of Göteborg, Sweden. He discovered a direct, strong bone anchorage of titanium chamber he was using while studying microcirculation in bone repair mechanisms. The titanium chamber was surgically inserted into the tibia of a rabbit. From additional information gathered in the study, the titanium was found to be the best material for artificial root replacement (Branemark *et al.* 1969, Albrektsson 1983, Zarb 1983, Branemark, 1983).

At the conclusion of the experiment, when it became time to remove the titanium chambers from the bone, it was discovered that the bone had integrated so completely with the implant that the chamber could not be removed and this phenomenon was described as "osseointegration" and it was seen the possibilities for human use. In dentistry the implementation of osseointegration started in the mid 1960s as a result of the work of Prof. Brånemark (Branemark 1983, Branemark *et al.* 1985, Albrektsson and Zarb 1989, John and Steven 1989).

In 1965 Brånemark placed dental implants into the first human patient named Gosta Larsson, inventing a new treatment option that would dramatically change the field of dentistry. This patient had a cleft palate defect and required dental implants to support an obturator. Gosta Larrson died in 2005, with the original implants still then in place after 40 years of function (McClarenc 2003, Wiki 2009, 2010a).

Despite the ongoing improvement in implant characteristics, bone intrinsic potential for osseointegration may be stimulated with adjuvant therapies to standard surgical procedures, as it is important to achieve the best possible implant osseointegration into the adjacent bone and to ensure therefore long-term implant stability. For this purpose, various pharmacological, biological or biophysical modalities have been developed, such as bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins (Dimitrio and Babis 2007).

Various topical growth factors studies have shown that platelet-rich plasma has become a valuable support for wound healing as an adjuvant therapy to standard surgical procedures. Autologous platelet-rich plasma represents a greater similarity to the natural healing process as a composite of multiple growth factors. It is safe due to its autologous nature and can be produced as needed from patient's own blood (Kathleen *et al*. 2010).

Platelet-rich plasma is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline. It also has been referred to as platelet-enriched plasma (PEP), platelet-rich concentrate (PRC), autologous platelet gel (APG), and platelet releasate (PR). Platelet rich plasma has been used to treat wounds since 1985. It

serves as a growth factor agonist and has both mitogenic and chemotactic properties. It contains a high level of platelets and a full complement of clotting and growth factors (Marx 2001, Pietrzak and Eppley 2005, Everts *et al.* 2006, Mehta and Watson 2008, Kathleen *et al.* 2010).

In humans, the typical baseline blood platelet count is approximately 2,000000 (2 million) per microlitre (μi); therapeutic PRP concentrates the platelets by roughly five-fold (Wiki 2011). A natural blood clot contains 95% red blood cells, 5% platelets, less than 1% white blood cells, and numerous amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% platelets, and 1% white blood cells (Anila and Nandakumar 2006, Wiki 2011).

The PRP functions as a tissue sealant and drug delivery system with the platelets initiating wound repair by releasing locally acting growth factors via α-granules de-granulation (Knighton *et al.* 1998). The secretory proteins contained in the α-granules of platelets include platelet-derived growth factor (PDGF-AA, BB, and AB isomers), transforming growth factor-β (TGF-β), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1) (Weibrich *et al.* 2001, Bhanot and Alex 2002, Gonshor A 2002, Henderson *et al.* 2003, Marx 2004, Pietramaggiori *et al.* 2006, Nikolidakis and Jansen 2008).

Platelets play a fundamental role in hemostasis and are a natural source of growth factors stored within platelet α-granules. The release of these

growth factors is triggered by the activation of platelets that can be initiated by a variety of substances or stimuli such as thrombin, calcium chloride, or collagen. Growth factors are involved in key stages of wound healing and regenerative processes including chemotaxis, proliferation, differentiation, and angiogenesis (Hom-Lay and Gustavo 2007).

These growth factors aid healing by attracting un-differentiated cells in the newly formed matrix and triggering cell division. Platelet rich plasma may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration. It promotes new capillary growth, and accelerates epithelialization in chronic wounds (Millington and Norris 2000, McAleer *et al.* 2006, Mishra *et al.* 2009).

Platelets in PRP also play a role in host defense mechanism at the wound site by producing signaling proteins that attract macrophages. PRP also may contain a small number of leukocytes that synthesize interleukins as part of a non-specific immune response. Previous studies on PRP have demonstrated antimicrobial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (S. aureus), including methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans*, and *Cryptococcus neoformans* (Tang *et al.* 2002, Lindeboom *et al.* 2007, Wrotniak *et al.* 2007, Bielecki *et al.* 2007).

Since PRP contains several growth factors that are capable to stimulate angiogenesis and increase fibroblast cell differentiation, using PRP to promote soft tissue healing has been proposed (Petrungaro 2001). Research showed that PRP and analogous products improve graft adhesion and minimizes micro-movement providing the most advantageous environment for graft acceptance (Whitman *et al.* 1997, Carlson and Roach 2002).

It has also been reported that PRP accelerates wound maturity and epithelialization, hence decreased scar formation. The platelet derived growth factors and epidermal growth factors (EGF) are the main growth factors involved in fibroblast migration, proliferation, and collagen synthesis. Increased concentrations of these growth factors are likely the reason for the accelerated soft tissue wound healing, which is suggested to be at least 2-3 times faster than that of normal (Anitua *et al.* 2004).

For the hard tissue, growth factors released from PRP are likely to effect on local vital cells such as osteoblasts. The addition of PRP to stromal cells has demonstrated angiogenic and osteogenic properties in animal models (Lucarelli *et al.* 2004)

Platelets are responsible for initiation of regeneration of tissue from trauma. During repair platelets become entrapped in a fibrin clot and degranulate releasing two primary growth factors: PDGF and TGF-B. The PDGF binds to endothelial cells to initiate capillary ingrowth, and TGF-B binds to osteoblasts and stem cells to initiate mitosis and stimulate osteoid production (Green 1998). The lifespan of platelets in a wound is less than five days. Macrophages are attracted into the graft site through an oxygen gradient of 30-40 mm Hg and drive the remaining bone regeneration process. By day 14, complete revascularization of the graft is seen. Stem cells differentiated into osteoblasts-osteoid is being laid down and early bone formation is occurs. By four to six weeks, random cellular bone, called woven bone, is formed which is immature and disorganized. In phase two remodeling lamellar bone is formed, representing a more organized bone (Anila and Nandakumar 2006).

Platelet rich plasma is easy to produce with minimal effort and can be prepared as needed at the point of care. In a two-step process, whole

blood from the patient is first centrifuged to separate the plasma from packed red blood cells and then further centrifuged to separate PRP from platelet-poor plasma. This concentrate is then activated with the addition of thrombin or calcium, resulting in a gelatinous platelet gel (Gandhi *et al.* 2005, Driver *et al.* 2006, Rozman and Bolta 2007).

Basically, patient's blood is collected and centrifuged at varying speeds until it separates into 3 layers: platelet poor plasma (PPP), PRP, and red blood cells. Usually 2 spins are used. The first spin ("Hard spin") separates the platelet poor plasma (PPP) from the red fraction and platelet rich plasma. The second spin ("Soft spin") separates the red fraction from the PRP. The material with the highest specific gravity (PRP) will be deposited at the bottom of the tube. Immediately prior to application, a platelet activator/agonist (topical bovine thrombin and 10% calcium chloride) is added to activate the clotting cascade, producing a platelet gel. The whole process takes approximately 12 minutes and produces a platelet concentration of 3-5x that of native plasma (Marx *et al.* 1998, Petrungaro 2001).

In humans, PRP has been investigated and used as clinical tool for several types of medical treatments, including nerve injury, tendinitis, chronic skin and soft tissue ulcerations, cardiac muscle injury, orthopedic and trauma surgery, cosmetic and plastic surgery, spinal surgery, heart bypass surgery, bone repair and regeneration, periodontal surgery, oral and maxillofacial surgery and burns (Mishra and Pavelko 2006, Aimetti *et al.* 2008, Griffin *et al.* 2009, Mishra *et al.* 2009, 2010, Por *et al.* 2009, Kathleen *et al.* 2010, Yu *et al.* 2011).

The use of PRP to enhance bone regeneration has been documented in periodontal defects, extraction sockets, during implant placement, and in guided bone regeneration procedures around implants, including sinus augmentation (Hom-Lay and Gustavo 2007, James *et al.* 2010, Manimaran and Saisada 2010).

1.2. RATIONALE

The rationale for using PRP in osseointegration of dental implant includes: reduce of post operative complication, stimulation of wound healing and enhancement of bone regeneration around dental implant providing the most advantageous environment for its acceptance. Advantages of using an autologous PRP also include no risk of cross reactivity, immune reaction or disease transmission (Home-Lay and Gustova 2007, Knighton *et al.* 1998, Kathleen *et al.* 2010, Wiki 2011).

The PRP is a new application of tissue engineering. Both the use and clinical validation of PRP is still in the early stages. The use of PRP to enhance bone regeneration around dental implant has been demonstrated by a few animal and human studies (Amany *et al.* 2006, Hesham *et al.* 2006, Anitua *et al.* 2007, Nazaroglou *et al.* 2009). Although the growth factors and mechanism involved are still poorly understood and many questions remain unanswered regarding the use of topical growth in wound healing. Ideal ratios of components of PRP are still being investigated, and the correlation between the concentration of PRP and the clinical effect is another area that needs to be investigated (Issa *et al.*, 2007). Even, the results of basic science and preclinical trials have not yet been confirmed in large scale controlled clinical trials (Foster *et al.* 2009).

Currently there is a paucity of critical scientific data regarding the beneficial effect of platelet rich plasma in clinical procedures. There have been animal and human studies both purporting and refuting its adjunctive positive effect (Amany *et al.* 2006, Hesham *et al.* 2006, Anitua *et al.* 2007, Marei *et al.* 2009, Nazaroglou *et al.* 2009). So, long term clinical study in this area is certainly needed.

The present study was designed to evaluate the effect of platelet rich plasma on osseointegration of dental implant. The further objective of this study was to compare the beneficiary impacts of PRP in implants patient. It was also projected to see the time needed for osseointegration of dental implants placed using platelet therapy and compare it with that of the implants placed without any adjuvant therapy. So, the present study was undertaken to document the clinical use with easy chair side technique, a risk free autogenous adjuvant therapy in addition to standard surgical procedure, benefits of the patients, and ultimately evaluation of its effects by clinical, radiographical and mechanical tools.

D-4178
27-03-2019

1.3. HYPOTHESIS

1.3.1. Alternative Hypothesis

Use of platelet rich plasma at the site of surgical placement of dental implant enhances osseointegration.

1.3.2. Null Hypothesis

Use of platelet rich plasma at the site of surgical placement of dental implant does not enhance osseointegration.

1.4. OBJECTIVES

1.4.1. General objective

This study was designed to evaluate the effect of platelet rich plasma on osseointegration of dental implant.

1.4.2. Specific objectives

Specific objectives of this study were:

- To evaluate the clinical success of osseointegration of dental implant placed with and without platelet rich plasma.
- To evaluate the imagistic values of implants placed with and without platelet rich plasma.
- To assess vertical bone loss at implant sites treated with and without platelet rich plasma.
- To assess horizontal bone loss at implant sites treated with and without platelet rich plasma.
- To measure the stability of implants treated with and without platelet rich plasma.
- To compare the outcome variables of implants placed with platelet rich plasma with those of placed without platelet rich plasma.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Human tooth and its loss

Teeth serve a basic physiologic function, beginning the process of digestion and nutrition through mastication and forming an essential part of gastrointestinal system. Teeth also play important role in speech, the mankind's primary form of communication.

Together with other oral and facial structures, it forms part of our appearance and is therefore an integral component of person's social, sensuous and psychological make-up. Tooth loss is thus not purely a functional or aesthetic deficit but also impacts an individual's overall quality of life. It may result in lowered self-confidence, altered self-image and altered behavior in socializing with as many as half the people surveyed reporting difficulties in coming to terms with their tooth loss (Fiske *et al.* 1998, 2001). So, teeth are an integral part of our social, sensuous and psychological well-being. Tooth loss leads to accentuation of facial crease and folds.

Fiske *et al.* (2001) studied the emotional effects of tooth loss in partially dentate people attending prosthodontic clinics in dental schools in England, Scotland and Hong Kong and found that forty nine per cent of all participants reported difficulties in accepting the loss of some of their teeth. People from Dundee were less likely to have difficulties accepting tooth loss. People from London took longer to come to terms with their tooth loss and were more likely to feel less confident. Fifty five per cent of all participants restricted their choice of foods and 54 per cent had not enjoyed their food as much as before. Fewer people in Dundee restricted their choice of food and were more likely to enjoy their food. People in Hong Kong were most likely to restrict their choice of food. Thirty five

percent of all subjects felt unprepared for the effects that tooth loss had upon them. People in Hong Kong were more prepared for tooth loss than those in Dundee and London. In addition, they were less concerned about leaving their dentures out overnight. They concluded that the emotional effects of tooth loss were significant in all groups, and people from London took longer to come to terms with their tooth loss.

Fiske *et al.* (1998) conducted another study where they found that the participants had a mean age of 69.9 years (range 51 to 86) and had been edentulous for a mean of 18.4 years (range 0.25 to 57 years). The main themes identified in reaction to tooth loss were bereavement, lowered self-confidence, altered self-image, dislike of appearance, an inability to discuss this taboo subject, a concern about prosthodontic privacy, behaving in a way that keeps the tooth loss secret, altered behaviour in socialising and forming close relationships, premature ageing, and lack of preparation. From their study they concluded that tooth loss can be disabling and handicapping. It has a profound impact on the lives of some people, even those who are apparently coping well with dentures. The profession needs to consider how it can prepare people for the effects of tooth loss.

A Dentist's Guide to Implantology (Ucer 2012) described that tooth loss has physiological effects that needs to be understood to provide suitable treatment. The most important of these is bone loss and atrophy. Tooth removal is the single most important factor underlying the loss of alveolar bone which is most pronounced during the first year of after tooth loss. Alveolar bone does not form in absence of teeth and this close association with presence of teeth and bone is maintained throughout life. A tooth is necessary for the development of alveolar bone and stimulation of bone is

necessary to maintain its load bearing capacity: density, quality and volume.

Craddol and Youngso (2004) studied of the incidence of overeruption and occlusal interference in unopposed posterior teeth. Their study showed the consequences of tooth loss. They reported that 83% of unopposed teeth were likely to overerupt, and the extent of the overeruption was marked. The incidence and extent of overeruption is of clinical significance, not only in terms of treatment planning to prevent undesirable vertical movement, but also in the restoration of the edentulous space. Moreover 51.6% of unopposed teeth are likely to be involved in premature contacts or excursive interferences. Both functional and anatomical changes that result from tooth loss and the consequent bone atrophy can have a knock-on effect on a patient's wellbeing or quality of life.

Rosenstiel *et al.* (2006) stated the consequences of tooth loss without replacement and they reported that the loss of posterior occlusion led to excessive forces on the remaining dentition with consequent damage and poor function. However, the studies demonstrated that adequate function was possible with reduced posterior occlusion. Deciding not to replace a tooth led to a situation in which the balance of the forces exerted on that tooth by the adjacent and opposing teeth and supporting tissues and by the soft tissues of the cheeks, lips, and tongue were upset. The consequences might be supraclusion of the opposing tooth or teeth, tilting of the adjacent teeth, and loss of proximal contact with resulting disturbances in the health of the supporting structures and the occlusion. However, the teeth adjacent to an edentulous space were not shown to be

at greater risk of damage, and the rate of change of teeth adjacent to an edentulous space was usually slow.

According to Andrewleon (2011) a tooth when is lost, the integrity of the dental arch is impaired. Loss of one or more teeth is known to disrupt the balance of the stomatognathic system and trigger several structural and functional changes. These include impaired chewing ability, changes in occlusal stability and occurrence of temporomandibular disorders (TMD).

Different studies have shown that tooth loss can have a substantial influence on the oral function (Sheiham *et al.* 2001, Nowjack and Sheiham 2003). However, although many epidemiologic studies express oral functionality by numbers of teeth, but it is questioned whether just the number of teeth is adequate to describe the functional status of the dentitions. It has been claimed that the occluding pairs of natural teeth are strongly correlated with oral functional status (Locker and Slade 1994). Besides the number of teeth also the teeth type, tooth location and number of occluding pairs determine the functionality (Gotfredsen and Walls 2007).

In a study on the impact of tooth loss on general health Florian *et al.* (2005) reported that reduced dentition without replacement of missing tooth by removable or fixed prosthodontics reduced the physical index of quality of life to the same extent as cancer and renal diseases.

Another study on tooth loss, chewing ability and quality of life stated that the chewing disabilities were related with the decrease of the number of natural teeth, therefore the oral health influenced the overall quality of life (Bortoluzzi *et al.* 2012).

There is no doubt that tooth loss can adversely affect a person's appearance. Patients seek dental treatment for both functional and esthetic or cosmetic reasons, and dentists have been successful in restoring or improving many patient's appearance. There is a list of the conspicuous and clinically challenging features that frequently accompany the edentulous state. These features include the facial morphological changes that are deepening of nasolabial groove, loss of labiodental angle, decrease in horizontal labial angle, narrowing of lips, increase in columella-philtral angle, and prognathic appearance (Zarb *et al.* 2005).

2.2. Replacement of Lost Tooth

Fixed partial dentures or bridges utilizing ceramics and alloys have been the mainstream for restoring small edentulous spans. However as in all forms of therapies, complications and failures occur (Goodacre *et al.* 2003). A lifespan of between seven and ten years is the norm with single crowns surviving longer than fixed partial dentures (FPDs) (9.1 years versus 7.7 years). Longer span bridges had a shorter survival rate with the exception of 6 unit canine to canine bridge which averaged 10.4 years (Walton *et al.* 1986). Based on a meta-analysis, Tan *et al.* (2004) calculated the 10 year probability of survival as 89.1% and the 10 year probability of success at 71.1%. When a cantilever was involved the respective 10 year probability of survival and success was 81.8% and 63% respectively (Pjetursson *et al.* 2004). In the study Walton *et al.* (1986) reported the average lifespan of a 2 unit cantilever bridge as 3.7 years.

Resin retained fixed partial dentures (adhesive bridges or resin retained bridges) have been indicated for long term interim restorations or when conservation of tooth structure is desired. Creugers and Hof Vant (1991)

carried out a meta-analysis of 1598 such bridges and reported a one year survival rate of 89% which declined linearly to 74% after 4 years. Removable dentures using base metals and acrylic have been used for large spans and also for economic reasons. Precision and semi-precision devices have been used to improve retention and stability of these prostheses.

Though the above options still have their place in dentistry, their current and future roles need to be reviewed in the light of recent advances in the field of implant dentistry and bone regeneration.

2.3. Implant Dentistry

Implant dentistry is an exciting and comparatively new field. Once thought of as a treatment modality of last resort, it has in recent years become integral part of mainstream dentistry as the old paradigms of what constitutes conservative dentistry are re-evaluated.

2.3.1. Definition of Dental Implant

Dental Implant is an alloplastic bicompatible material surgically inserted into the soft and hard tissue of the jaws primarily as prosthodontic foundation, thereby providing support and retention of dental prosthesis (Misch 2011). It is a unique modality to replace the missing tooth.

A prosthetic superstructure is subsequently fitted onto trans-epithelial posts or abutments joined to the buried implants. The successful insertion of a biocompatible material into living tissue has revolutionized medicine and dentistry. There is no evidence of rejection of implants but a little negligible failure rate is reported. Today, there are ever increasing demands from patients with missing teeth for the restoration of their

masticatory function and aesthetic appearance with dental implants (Zarb *et al.* 2005, Matusovits 2009).

2.3.2. History and Concept

The concept of using dental implants to replace missing teeth dates back to the early Egyptians, where archaeological findings, such as carvings of tooth replicas (circa 5500 BC) and empirical implants in mummies, were found in burial sites (circa 2500 BC). Alloplastic implants could date as far back as around 600 AD in which artifacts of Maya civilisation apparently but inconclusively showed radiographic evidence of bone in apposition with shell implants. Besides, ancient skulls discovered by means of archaeological findings have shown objects such as stones and seashells serving as replacement to the function of natural teeth. Furthermore, some of these foreign materials were shown to display actual fusion to the alveolar bone. Much has changed since the Mayan attempt at creating a dental implant (Irish 2004).

The dental implant has a lengthy history, beginning with ancient Egiptians, who implanted teeth in corpses in accordance with religious beliefs regarding the afterlife. According to evidence discovered in underground burial chamber in what is now modern Italy, early Eutruscans replaced missing teeth with artificial teeth carverd from bone of oxen. The Romans conquered the Eutruscans and employed their dental techniques until the fall of Rome. The earliest endosseous implant was in a mandible fragment of Mayan origin dating from about A. D. 600. Radiographs showed compact bone formation around the three tooth-shaped pieces of shell implanted in sockets of missing lower incisors, similar to the bone surrounding a modern blade implant (Ring 1995a).

Innovation of dentistry dwindled following the fall of the Roman Empire, but they were revived during Renaissance. By the 1800s, fixed bridges and partial dentures were successful methods of tooth replacement. In 1885, Dr. J. M. Younger implanted a natural human tooth into artificial socket. The procedures included filling the pulp chamber of the tooth with gutta percha and the apical opening with gold. A tooth from any source was acceptable provided that the asepsis was maintained. Although his work was largely unsuccessful, it spurred many later attempts at implantation. Technical advances include implanted tubes of gold and radium, lead and porcelain posts, and bovine incisor teeth into natural and artificially created sockets (Ring 1995a).

In 1948, two American dentists, Gershop and Goldberg, surgically placed a subperiosteal implant created by Dr. Gutav Dahl of Sweden. The subperiosteal implant was prefabricated based on a study model. This method of implantation met with limited success and proved over time to have a high failure rate due to infection (Ring 1995a).

In 1965, Swedish orthopedist P. I. Brånmark placed the first titanium implant and coined the term "osseointegration" (Ring 1995b). Osseointegration-incorporation of the implant with the bone is one of the greatest achievements in implant dentistry. In 1967, Dr. Leonard Linkow of New York City placed the first blade implant, and by the 1970s, this was the most frequently employed implant design (Ring 1995b). In recent years it has become a highly predictable and unique treatment modality to replace missing teeth (Zarb *et al.* 2005, Misch 2011).

2.4. Osseointegration

2.4.1. Definition

Osseointegration, or predictable long-term anchorage of tooth root analogues in bone, is defined as "a time dependent healing process whereby clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading" (Zarb and Albrektsson 1991). Osseointegration refers to a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant. Osseointegrated implant provides a foundation to support prosthesis and has the ability to transmit occlusal forces directly to bone. In this phenomenon/process the implant must be made of an inert biocompatible material to be in direct contact with bone; without soft tissue, scar tissue, cartilage or ligament interface. Currently, an implant is considered as osseointegrated when there is no progressive relative movement between the implant and the bone with which it has direct contact. When Osseointegration occurs, the implant is tightly held in place by the bone developing a close bond between the two. In general, it is the wound healing with bone regeneration around dental implant after its surgical placement. The process typically takes four to six months to occur (Albrektsson *et al.* 1981, Branemark 1983, Carlsson *et al.* 1986, Mavrogenis *et al.* 2009, Nandal *et al.* 2014).

This phenomenon of "osseointegration" is characterized by a number of clinical and ultrastructural observations. Osseointegration may broadly be defined as the dynamic interaction and direct contact of living bone with a biocompatible implant in the absence of an interposing soft tissue layer (Albrektsson *et al.* 1983, Branemark *et a.*1985, Masuda et al. 1998).

The biological fixation between the dental implant and jaw bones should be considered a prerequisite for the long-term success of implant supported prostheses (Arthur *et al.* 2010). The long-term clinical success of dental implants is related to their early osseointegration (Sandrine *et al.* 2010).

2.4.2. History of Osseointegration

Implant dentistry today owes much to the work of Brånemark and his co-workers whose classical work on osseointegration described the relationship between bone and implant as visualized histologically. The concept of osseointegration was first developed and the term was coined by orthopaedic surgeon Dr. Per-Ingver Branemark, Professor at The Institute for Applied Biotechnology, University of Göteborg, Sweden. He discovered a direct, strong bone anchorage of titanium chamber he was using while studying microcirculation in bone repair mechanisms. The titanium chamber was surgically inserted into the tibia of a rabbit. From additional information gathered in the study, the titanium was found to be the best material for artificial root replacement (Branemark *et al.* 1969, Albrektsson 1983, Zarb 1983, Branemark 1983).

At the conclusion of the experiment, when it became time to remove the titanium chambers from the bone, it was discovered that the bone had integrated so completely with the implant that the chamber could not be removed and this phenomenon was described as "osseointegration" and it was seen the possibilities for human use. In dentistry the implementation of osseointegration started in the mid 1960s as a result of the work of Prof. Brånemark (Branemark 1983, Branemark *et al.* 1985, Albrektsson and Zarb 1989, John and Steven 1989).

In 1965 Brånemark placed dental implants into the first human patient named Gosta Larsson, inventing a new treatment option that would dramatically change the field of dentistry (McClarenc, 2003). This patient had a cleft palate defect and required dental implants to support an obturator. Gosta Larrson died in 2005, with the original implants still in place after 40 years of function (Wiki 2009, 2010a, b).

Interest in dental implants accelerated after a landmark study conducted by Adell *et al.* (1981). In that study, a total of 2768 fixtures were placed in 410 jaws of 371 consecutive patients. The success rate of fixtures was 81% in the maxilla and 91% in the mandible. For prosthetic success, the figures were 91% and 100% respectively.

2.4.3. Biology of Osseointegration

Although the clinical term osseointegration describes the anchorage of endosseous implants to withstand functional loading, it provides no insight into the mechanisms of bony healing around such implants. However, it is clear that the long-term success of dental implants also depends on the complex biointegration of these alloplastic materials, which is determined by the responses of the different surrounding host tissues like the alveolar bone, the conjunctival part of the oral soft tissues and the gingival epithelium. Nevertheless, an understanding of the sequence of bone-healing events around endosseous implants is believed to be critical in developing biologic design criteria for implant surfaces. Bone growth on the implant surface can be phenomenologically subdivided into three distinct phases that can be addressed experimentally (Davies 1998).

The first, osteoconduction, relies on the migration of differentiating osteogenic cells to the implant surface, through a temporary connective

tissue scaffold. Anchorage of this scaffold to the implant surface is a function of the implant surface design. The second, *de novo bone formation*, results in a mineralized interfacial matrix, equivalent to that seen in cement lines in natural bone tissue, being laid down on the implant surface. The implant surface topography determines whether the interfacial bone formed is bonded to the implant. A third tissue response, the bone remodelling, creates a bone-implant interface comprising *de novo* bone formation. Treatment outcomes in dental implantology depend critically on the implant surface designs that optimize the biological response during each of these three distinct integration mechanisms (Davies 1998).

Bone healing around implants involves the activation of a sequence of osteogenetic, vascular and immunological events that are similar to those occurring during bone healing (Soballe 1993). Various cell types, growth factors and cytokines are involved and interact throughout the stages of osseointegration, including inflammation, vascularisation and bone formation and ultimately bone remodeling (Linder *et al.* 1989). The primary host response after implantation is an inflammatory reaction elicited by the surgical trauma and modified by the presence of the implant. Initially, a haematoma is formed at the bone-implant interface and may play a role as a scaffold for peri-implant bone healing (Park and Davies 2000).

The host response consists of platelet activation, migration and activation of inflammatory cells, vascularization, mesenchymal cells and osteoblast adhesion, proliferation, protein synthesis, and local factor composition (Nygren *et al.* 1997, Davies 1998, Park and Davies 2000, Boyon *et al.* 2002). From the implant side, an oxidation of metallic implants has been

observed (Sundgren *et al.* 1985). Osteoblasts also attach on the implant surface from day one of implant insertion (Meyer *et al.* 2004). Furthermore, the deposition from osteogenic cells on the implant surface of a layer of non-collagenous proteins that regulate cell adhesion and binding of minerals has been described during the early stages of host response (Albrektsson and Hansson 1996). A few days after implantation, osteoblasts begin to deposit collagen matrix either in direct contact with the implant surface (Meyer *et al.* 2004) or directly on the early afibrillar interfacial zone comparable to cement lines, which is rich in non-collagenous proteins such as osteopontin and bone sialoprotein (Puleo and Nancyn 1991).

The early deposition of new calcified matrix is followed by woven bone formation to ensure tissue anchorage and ultimately is substituted by lamellar bone, thus completing the biological fixation of the implant (Chappard *et al.* 1999). Peri-implant osteogenesis progresses either from the host bone towards the implant surface which is known as distance osteogenesis or from the implant towards to the healing bone known as contact osteogenesis or *de novo* bone formation (Davies 1998).

Vascularisation is essential during osseointegration, as it influences tissue differentiation and ossification (Marco *et al.* 2005). Bone remodelling ultimately occurs for reshaping or consolidation of bone at the implant site, providing a mechanism for self repair and adaptation to stress. Overall osseointegration of implants in humans is a slow process and can take up to several months (Kim and Kim 1993, Hofmann *et al.*1997).

2.4.4. Factors Enhancing Osseointegration

Despite the ongoing improvement in implant characteristics, bone intrinsic potential for osseointegration may be stimulated with adjuvant therapies to standard surgical procedures, as it is important to achieve the best possible implant osseointegration in the shortest possible healing time and to ensure therefore long-term implant stability (Dimitriou and Babis 2007).

Bone implant osseointegration depends on several factors which can be divided into different groups such as: i) implant-related factors, ii) the status of the host bone bed, iii) the mechanical stability and iv) the use of adjuvant therapies (Dimitriou and Babis 2007).

2.4.4.1. Implant-related Factors

According to Marco *et al.* (2005) the implant related factors include implant material, implant design, chemical composition of implant, topography of the implant surface. The different materials, shape, length, diameter, implant surface treatment and coatings have been proposed to enhance clinical performance.

The biocompatibility of the material is of great importance and a predictor of osseointegration, as it is essential to establish stable fixation with direct bone-implant contact and no fibrous tissue at the interface (Anselme 2000). Pure Titanium (Ti) is a widely used implant material as it is highly biocompatible, it has good resistance to corrosion, and no toxicity on macrophages or fibroblasts, lack of inflammatory response in peri-implant tissues and its surface is composed of an oxide layer and has the ability to repair itself by reoxidation when damaged (Rae 1975, 1981, Brune *et al.* 1982, Browne and Gregson 2000, Breme *et al.* 1988). Other

materials have also been proposed either as alternative to Ti or as alloy systems, including tantalum, aluminium, nionium, nickel, zirconium, and hafnium (Alberius 1983, Johansson and Albrektsson 1991, Thomsen *et al*. 1997, Mohammadi *et al.* 2001, Kujala *et al*, 2003).

The most frequent implant surfaces and types can be subdivided into implants with roughened surfaces with a coating, e.g., titanium plasma-sprayed or hydroxyapatite coated; implants with machine-processed titanium without a coating, e.g., machined or polished; and implants with roughened surfaces without a coating e.g., sand-blasted, acidetched or anodically roughened (Cochran *et al.*1996, Larsson *et al.* 1996, Kurzweg *et al.* 1998, Soballe *et al.* 1999).

Rough surfaces enlarge the implant area in contact with the host bone favouring primary stability, and enhancing peri-implant bone formation compared to smooth surfaces (Cochran *et al*. 1996). Roughness positively affects osseointegration (Borsari *et al.* 2005) and, in particular, it seems to affect directly osteoblast attachment and subsequent proliferation and differentiation (Fini and Giardino 2003). In general, moderately rough surfaces favour peri-implant bone growth better than smoother or rougher surfaces (Albrektsson and Wennerberg 2004).

The pore size of a porous coated implant seems to be a major determinant of osseointegration (Bobyn *et al*. 1980). Among different sizes, a pore size above 80 μm improves bone ingrowth in both hydroxyapatite and tricalcium phosphate materials (Galois and Mainard 2004)

2.4.4.2. The Implant Host Site and Its Healing Potentiality

A high quality healthy bone, minimum surgical trauma, bone cellularity and vascularity influence the primary stability of implant that leads to influeinfluence the bone implant osseointegration (Linder *et al.* 1989).

A healthy bone bed with minimal surgical trauma is important as it is the source of almost all cells, local regulatory factors, nutrients, and vessels that contribute to the bone healing response. The implantation site influences the osseointegration process through different levels of bone cellularity and vascularity. A high-quality bone also seems to be important for the initial implant stability. Other factors, such as the geometry and size in the case of recipient defect sites, have also been reported to influence bone implant osseointegration (Spadora *et al.* 1990, Wittenberg *et al.* 1991, de Vicente *et al.* 2006).

2.4.4.3. The Mechanical Stability

Primary implant stability consists in rigid fixation between the implant and the host bone cavity with no micro-motion of the implant or minimal distorsional strains. Primary stability depends on the surgical technique, the implant design, and the implantation site. In cortical bone a higher mechanical anchorage to the implant is observed compared to cancellous bone (Sennerby *et al.* 1992, Soballe 1993, Giori *et al.* 1995, Branemark *et al.* 1977).

2.4.4.4. Use of Adjuvant Therapies

Despite the ongoing improvement in implant characteristics, bone intrinsic potential for osseointegration may be stimulated with adjuvant therapies to standard surgical procedures, as it is important to achieve the best possible implant osseointegration into the adjacent bone and to

ensure therefore long-term implant stability. For this purpose, various pharmacological, biological or biophysical modalities have been developed, such as bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins (Dimitriou and Babis 2007).

It is revealed from the various studies that platelet-rich plasma has become a valuable support for wound healing as an adjuvant therapy to standard surgical procedures. Autologous platelet-rich plasma represents a greater similarity to the natural healing process as a composite of multiple growth factors. It is safe due to its autologous nature and can be produced from patient's own blood (Kathleen *et al.* 2010).

2.5. Platelet Rich Plasma

2.5.1. Definition and Composition

Platelet-rich plasma is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline. It has also been referred to as platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel, and platelet releasate. Platelet rich plasma has been used to treat wounds since 1985. It serves as a growth factor agonist and has both mitogenic and chemotactic properties It contains a high level of platelets and a full complement of clotting and growth factors (Marx 2001, Pietrzak and Eppley 2005, Everts *et al.* 2006, Mehta and Watson 2008).

In humans, the typical baseline blood platelet count is approximately 20,00,000 (2 million) per microlitre (μL); therapeutic PRP concentrates the platelets by roughly five-fold (Wiki 2011). A natural blood clot contains 95% red blood cells, 5% platelets, less than 1% white blood

cells, and numerous amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% platelets, and 1% white blood cells (Anila and Nandakumar 2006, Wiki 2011).

2.5.2. Mechanism of Action of Platelet Rich Plasma

Platelet rich plasma functions as a tissue sealant and drug delivery system with the platelets initiating wound repair by releasing locally acting growth factors via α-granules de-granulation (Knighton *et al.* 1998). These growth factors aid healing by attracting un-differentiated cells in the newly formed matrix and triggering cell division. These may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration, promote new capillary growth, and accelerate epithelialization in chronic wounds (Millington and Norris 2000, McAleer *et al.* 2006, Mishra *et al.* 2009).

Platelets in PRP also play a role in host defense mechanism at the wound site by producing signaling proteins that attract macrophages. It also may contain a small number of leukocytes that synthesize interleukins as part of a non-specific immune response. Previous studies have demonstrated antimicrobial activity of PRP against *Escherichia coli, Staphylococcus aureus* including methicillin-resistant *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans* (Tang *et al.* 2002, Lindeboom *et al.* 2007, Wrotniak *et al.* 2007, Bielecki *et al.* 2007).

Platelets play a fundamental role in hemostasis and are a natural source of growth factors. The release of these growth factors is triggered by the activation of platelets that can be initiated by a variety of substances or stimuli such as thrombin, calcium chloride, or collagen. Growth factors are involved in key stages of wound healing and regenerative processes

including chemotaxis, proliferation, differentiation, and angiogenesis (Hom-Lay and Gustavo 2007).

The growth factors (GFs) are present at increased concentrations in PRP. In addition to growth factors, platelets release numerous other substances (e.g., fibronectin, vitronectin, sphingosine 1-phosphate, etc.) which are important in wound healing. An advantage of PRP over the use of single recombinant human growth factor delivery is the release of multiple growth factors and differentiation factors upon platelet activation (Sanchez *et al.* 2003). The PRP is a fibrin framework over platelets that has the potential to support regenerative matrix (Fernandez *et al.* 2006, El-Sharkawy *et al.* 2007)

2.5.3. Applications of Platelet Rich Plasma

The PRP is used in different treatments which include chronic skin and soft tissue ulcerations, periodontal and oral surgery, maxillofacial surgery, orthopedic and trauma surgery, cosmetic and plastic surgery, spinal surgery, heart bypass surgery, and burns (Kathleen *et al.* 2010).

The use of PRP to enhance bone regeneration has been documented in periodontal defects, extraction sockets, during implant placement, and in guided bone regeneration procedures around implants, including sinus augmentation (Hom-Lay and Gustavo 2007, James *et al.* 2010, Manimaran and Saisada 2010).

According to Georgakopoulos *et al.* (2014) PRP augments the osteo-regenerative potential of surrounding tissues after dental implanting, which in turn orient the daily surgical procedure towards PRP employment. They investigated the temporal texture differentiation associated with the bone regeneration properties, around loaded implants,

after platelet rich plasma (PRP) application in a follow up clinical sample of panoramic radiographs by means of the differentiation of the image texture, and reported that the addition of the PRP had a significantly positive effect on bone formation as captured by dental panoramic radiographs.

2.5.3.1. Application in Regenerative Activity

2.5.3.1.1. On Tooth Supporting Structures

Regeneration of tooth-supporting structures destroyed by periodontitis is a major goal of periodontal therapy. Periodontal regeneration is perhaps one of the most complexes to occur in the body since at least six tissues are involved viz., the gingival epithelium, gingival connective tissue, periodontal ligament, tooth root surface cementum, alveolar bone and corresponding vasculature. All these mineralized and nonmineralized components must be restored to their original position and architecture for regeneration of the periodontium to occur. Growth factors are a class of naturally occurring proteins involved in three key cellular events in tissue repair: mitogenesis, migration and matrix synthesis and remodeling1. A combination of these growth factors may more effectively stimulate formation of mineralized as well as nonmineralized tissues. Platelets are rich in growth factors that may contribute to an accelerated tissue regeneration process (Samule 1994, Navins *et al.* 2005, Anila and Nandakumar 2006).

2.5.3.1.2. On Sequence of Bone Regeneration

Platelets are responsible for initiation of regeneration of tissue from trauma. During repair platelets become entrapped in a fibrin clot and degranulate releasing two primary growth factors viz., PDGF and TGF-B. The PDGF binds to endothelial cells to initiate capillary ingrowth; and

TGF-B binds to osteoblasts and stem cells to initiate mitosis and stimulate osteoid production (Green 1998). The lifespan of platelets in a wound is less than five days. Macrophages are attracted into the graft site through an oxygen gradient of 30-40 mm Hg and drive the remaining bone regeneration process. By day 14, complete revascularization of the graft is seen. Stem cells are differentiated into osteoblasts, osteoid being laid down, and early bone formation occurs. By four to six weeks, random cellular bone, called woven bone, is formed which is immature and disorganized. In phase two remodeling lamellar bone is formed, representing a more organized bone (Anila and Nandakumar 2006).

2.5.4. Rationale to Use RPP Rather Than Other Adjuvant Therapies

As an autologous preparation, PRP is safer to use than allogenic or homologous preparations and is free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever, and Creutzfeldt-Jakob disease. It requires no special considerations regarding antibody formation, effectively preventing the risk of graft vs. host disease and leading to better acceptance by patients (Kathleen *et al.* 2010).

The rationale for using PRP in soft and hard augmentation are to accelerate vascularization of the graft, improve soft tissue healing, reduce post operative morbidity, and enhance bone regeneration (Anitua 1999). Advantages of using an autologous PRP include no risk of cross reactivity, immune reaction or disease transmission (Weibrich *et al.* 2001). In addition, the use of PRP improves handling of graft materials and easier packing into a grafting site, thus facilitating space maintenance and potential bone regeneration (Jakse *et al.* 2003, Freymiller and Aghaloo 2004).

Since PRP contains several growth factors that are capable to stimulate angiogenesis and increase fibroblast cell differentiation, using PRP to promote soft tissue healing has been proposed (Petrungaro 2001). Research showed that PRP and analogous products improve graft adhesion and minimizes micro-movement, providing the most advantageous environment for graft acceptance (Whitman *et al.* 1997, Carlson and Roach 2002). The PRP accelerates wound maturity and epithelialization, hence decreased scar formation. Both PDGF and EGF are the main growth factors involved in fibroblast migration, proliferation, and collagen synthesis. Increased concentrations of these growth factors are likely the reason for the accelerated soft tissue wound healing, which is at least 2-3 times faster than that of normal (Anitua *et al.* 2004).

For the hard tissue, growth factors released from PRP are likely to effect on local vital cells such as osteoblasts. The addition of PRP to stromal cells has demonstrated angiogenic and osteogenic properties in animal models (Lucarelli *et al.* 2004).

One of the major drawbacks of bone augmentation is the extended healing time required. Hence, one of the major reasons proposed for the use of PRP is a reduced healing time. A shortened graft healing time (50%) has been demonstrated in sinus augmentation. Accelerated bone regeneration has also been demonstrated in periodontal defects distal to second molars when PRP is added at the time of extraction of impacted third molars. Unfortunately, these results cannot be used to expound the beneficial effects of PRP, as biopsies were not taken from any of the control sites (Kassolis and Reynolds 2005, Sammartino *et al.* 2005).

2.6. Procurement of Platelet Rich Plasma

Platelet rich plasma can be obtained in various ways in the dental office. Techniques for PRP preparation vary from using 10 cc of a patient's blood and spinning it in a lab centrifuge, to using a unit of blood that is put through a cell separator that sequesters and concentrates the platelets (Marx 1999).

2.6.1. Systems for Procurement

2.6.1.1. One Touch Automated PRP Systems

It provides simplicity in operation and may provide a good platelet count before plasma resuspension. This system requires 50 ml blood for procurement and do not sense the Plasma/Blood Interface and hence may yield low platelet count

2.6.1.2. Plasmapheresis

It requires approximately 450 ml of blood from which 20-60 cc of PRP is obtained. This method of cell separation is used only when large quantities of PRP are required. The method requires sophisticated equipments and laboratory (Westphal 1984).

2.6.1.3. Manually PRP Preparation

End user can manually recover the maximum amount of platelets. This is the best way to produce a true PRP product. This system facilitates to achieve the concentration up to counts of 4 to10 times the patient's baseline PRP. Since there is no re-suspending (dilution) of platelets, the final product has a high concentration of platelets.

2.6.2. General Preparation and Activation of Platelet Rich Plasma

Platelet rich plasma is easy to produce with minimal effort and can be prepared as needed at the point of care. In a two-step process, whole blood from the patient is first centrifuged to separate the plasma from packed red blood cells and then further centrifuged to separate PRP from platelet-poor plasma. This concentrate is then activated with the addition of thrombin or calcium resulting in a gelatinous platelet gel. Clinically valuable PRP contains at least one million platelets per microliter. Lesser concentrations cannot be relied on to enhance wound healing, and greater concentrations have not been shown to increase wound healing (Marx 2001, Mishra *et al.* 2009).

Predictable and efficient compact systems to develop PRP can be used in both office and hospital settings. While medical practitioners are able to apply blood products in the office, as is done with PRP, they are not licensed to infuse or re-infuse blood or blood products in an office setting. Because PRP producing systems only require a small amount of blood to produce, there is no need for reinfusion, and studies have shown that these frequent but small blood draws do not have an effect on hemoglobin, hematocrit, or platelet count (Marx 2004, Driver *et al.* 2006).

Not all currently marketed PRP devices are equivalent because not all concentrate viable platelets in sufficient numbers to enhance healing, with these differences accounting for many of the criticisms regarding the efficacy of PRP (Marx 2004). Although there is a wide range of devices for the preparation of PRP, not all have been approved for use in humans. The only autologous PRP separation system currently indicated for use in diabetic ulcers is the AutoloGel™ System (Cytomedix, Inc., Rockville,

MD), which contains all materials, including bovine thrombin, necessary to activate the PRP gel and can be used by health care providers without specialized technicians (Mazzucco *et al.* 2008, AutoloGel System 2009, Kathleen *et al.* 2010).

2.6.3. A Simple Chair Side Technique for PRP Preparation

Recent publications have indicated that PRP prepared from 8 to 10 ml of whole blood is sufficient for periodontal regenerative therapies (Weibrich *et al.* 2001). Clinicians can procure it with the help of a general purpose tabletop laboratory centrifuge by the following method. It is simple and cost-effective method for producing PRP in an in-office environment. Patients are selected based on the absence of any blood abnormalities or use of anti-coagulants. Ten millimeter (10 ml) blood is withdrawn from the anticubital region with a 10ml syringe and transferred to a container containing 1.4ml anticoagulant citrate phosphate dextrose solution (CPDS). It is then centrifuged for 10 minutes at 1300 rpm. The result is a separation of whole blood into a lower red blood cell (RBC) region and upper straw-colored plasma region as shown in fig B. There is relatively high concentration of platelets found in the boundary layer between these two regions. The upper straw colored platelet poor plasma (PPP) layer and 1-2 mm of the top part of the RBC layer is aspirated and transferred into another container and again centrifuged for 10 minutes at 2000 rpm. This results in an upper portion of clear yellow supernatant serum and the

bottom red tinged layer consisting of highly concentrated PRP. Leaving 1.5 mm, rest of the upper clear layer is aspirated. The contents of the tube is mixed well and transferred into a sterile container. At the time of the application, the PRP is combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate). This results in formation of a sticky gel that is relatively easy to apply to the surgical sites (Anila and Nandakumar 2006, Kathleen *et al.* 2010). The steps of PRP preparation has been shown in appendix 18.

2.7. Use of Platelet Rich Plasma (PRP) in Implant Surgery

Platelet-rich plasma is a new approach to tissue regeneration and it is becoming a valuable adjunct to promote healing in many procedures in dental and oral surgery, especially in aging patients. It is derived from the centrifugation of the patient's own blood and it contains growth factors that influence wound healing, thereby playing an important role in tissue repairing mechanisms. The use of PRP in surgical practice could have beneficial outcomes, reducing bleeding and enhancing soft tissue healing and bone regeneration. The previous studies on humans have yielded promising results regarding the application of PRP to many dental and oral surgical procedures i.e. tooth extractions, periodontal surgery, implant surgery (Antonino *et al.* 2013).

Some selected references on the RCTs using PRP in soft/bone tissue surgery and implant surgery are shown in Table 1.

Table 1: Some selected references on the RCTs using PRP in soft/bone tissue surgery and implant surgery.

Authors	Number of patients	Treatment	Follow-up (wks)	Main results	Effect of PRP
Anitua *et al.* (2006)	295	Implantology	8	Improvement in implant prognosis	strong
Anand and Mehta (2012)	11	Implantology	12-24-36-48	Improved early bone apposition around the implant	strong
Gentile *et al.* (2010)	15	Reconstructive surgery of the jaw	2-4-12-24	Efficacy of PRP treatment in terms of patient satisfaction and low-morbidity	strong
Wojtowicz *et al.* (2007)	16	Augmentation of mandibular bone	12	PRP is more effective than bone marrow, containing CD34+ cells	strong
Daif (2012)	24	Bone regeneration of mandibular fractures	1-12-24	Direct application of the PRP along the fracture lines may enhance bone regeneration in mandibular fractures	strong
Khairy *et al.* (2012)	15	Sinus lift	12-24	PRP- enriched bone grafts were associated with superior bone density at 6 months post grafting	strong
Poeschl *et al.* (2012)	14	Sinus lift	28	Increased new bone formation when PRP was used	strong
Cabbar *et al.* (2011)	10	Sinus lift	28	No statistically significant differences were observed	Weak

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Design of Study

This was an interventional prospective controlled clinical trial.

3.2. Place of Study

The clinical part of this research was carried out in Face-bow Institute of Implant Dentistry & Prosthodontics, Mirpur, Dhaka; and Anwer Khan Modern Medical College & Hospital, Dhanmondi, Dhaka.

3.3. Duration of Study

The research work was carried out from August 2012 to December 2016.

3.4. Study Population

People having missing teeth required replacement with implant supported prostheses were the population of this research.

3.5. Sampling Technique

Consecutive sampling technique was used to select the sample of this study.

3.6. Sample Size

Total evaluated sample size was 288 implants, of them 144 implants were placed using platelet rich plasma as an adjuvant therapy and considered as study group, and other 144 implants were placed conventionally without using any adjuvant therapy and considered as control group.

3.7. Sample Size Determination

The sample size of the study was calculated by the following formula.

$$\mathbf{n} = \frac{(\mathbf{u}+\mathbf{v})^2(\sigma_1^2+\sigma_2^2)}{(\mu_1-\mu_2)^2}$$

Here,

μ_1= mean of one group, σ_1= SD of one group (from previous study)

μ_2= mean of other group, σ_2= SD of other group (from previous study)

u = value of standard normal distribution at a given level of significance

v = value of standard normal distribution at a given study power

n = sample size of each group (Hoque 2016).

Accordingly, here, μ_1=70, σ_1=7, μ_2= 73, σ_2=8, u=1.28 at 5% level of significance and v = 1.96 with 90% study power.

Mean values (μ_1 & μ_2) and standard deviations (σ_1 & σ_2) were taken from the previous study conducted by Gailani and Lateef (2015).

So,

$$\mathbf{n} = \frac{(1.28+1.96)^2(7^2+8^2)}{(70-73)}$$

$$= \frac{10.5 \times 113}{9}$$

$$=131.83\approx132$$

According to the formula the calculated sample size for each group was 132. This number was increased to 150 in each group to make allowance for incomplete or missing data and drop out of the participants from the study. So, a total 300 patients were selected and treated in the study and ultimately 288 patients (144 in study group and 144 in control group) were evaluated for presenting results.

3.8. Ethical Consideration

The project proposal of this study entitled "EFFECT OF PLATELET RICH PLASMA ON OSSEOINTEGRATION OF DENTAL IMPLANT" was approved by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) of Institute of Biological Sciences of University of Rajshahi in its Resolution No. 5 of the 5th meeting held on 26th December 2013 (Appendix 4).

Patients participated in this study were consented prior to participation after detail explanation of treatment steps. Informed consent form was signed by each patient after reading, understanding and realizing the merits and demerits of implant treatment (Appendix 2).

Every patient was assured about the confidentiality and freedom to withdraw himself/herself from the study at any time.

Each participant was also assured about the adequate treatment of any complications developed in relation to the study procedure.

3.9. Patient Selection

The general physical condition of each patient was evaluated accurately in order to obtain an overall health assessment. Initial data were obtained from each patient through medical history, dental history, radiographic study, casts analysis, and photograph. In addition, a thorough clinical examination was done to assess the soft tissues and contour of the edentulous space as well as adjacent natural teeth if the area was fit for implant placement. The existing occlusion was assessed for future implant supported prosthesis.

3.9.1. Inclusion Criteria

Patients having following criteria were included in this study:

- Patient aged above 18 years
- Partially edentulous patient with single missing tooth
- Edentulous site was evident with minimum amount of bone volume for placement of implant
  - » height: 10 mm
  - » width: 6 mm
  - » length: 7 mm
- Absence of systemic contraindications were evident with
  - » metabolic disorders
  - » immunodeficiency
  - » haematological diseases
  - » neoplastic diseases
  - » bisphosphonates history and
  - » smoking (>10 cigarettes/day)
- Absence of local contraindications were evident with
  - » head and neck radiotherapy
  - » poor oral hygiene
  - » active periodontal disease
  - » parafunctions e. g. bruxism
  - » nonalcoholic
- Patients psychologically against removable prosthesis
- No anatomical limitations for placement of implant
- Patients committed to maintain good oral hygiene
- Had some knowledge and trust of dental implant treatment

3.9.2. Exclusion Criteria

Patients having following criteria were excluded from the research work:

- Patient aged below 18 years
- Irradiated patient
- Psychiatric problem
- Haematologic disorders
- Existing pathology of hard or soft tissue
- Recent extraction
- Total edentulism
- Drug, alcohol, or tobacco abuse
- Uncontrolled diabetes
- Congenital or acquired heart defect patient
- Ischemic heart disease (angina, recent myocardial infarction)
- High uncontrolled blood pressure
- Inadequate upper/lower posterior height
- Inadequate lower anterior width
- Extremely poor bone substance
- Patient's distrust of implant treatment
- Metabolic disorders
- Immunodeficiency
- Neoplastic diseases
- Bisphophonates history and
- Smoking (>10 cigarettes/day)
- Head and neck radiotherapy
- Poor oral hygiene
- Active periodontal disease
- Parafunctions e. g. bruxis
- Pregnant women

3.10. Clinical Examination

Each patient was clinically evaluated while seated on a well equipped and illuminated dental chair. Extra oral examination was done to evaluate any asymmetry, deformity and defect in face, nose, ear, eyes, lips and cheeks. Intra oral examination was done using dental mirror and probe under well lit condition to evaluate each of the remaining teeth and its supporting structures. The detail status of oral hygiene, masticatory pattern and habit of each patient were accurately evaluated and noted. The edentulous space specific for site of implant placement was examined in terms of soft tissue condition, bony contour, width, length, and inter arch space.

3.11. Diagnostic Cast Analysis

Diagnostic impressions of both arches for each patient were made with standard methods and materials. Diagnostic casts were fabricated with dental stone from the impressions for the purpose of study/analysis. On the day of impression taking occlusal registration of each patient was recorded with silicon impression/registration materials. The casts were mounted on semi-adjusted articulator for analysis.

The remaining dentitions, edentulous space, and maxillomandibular relationship were evaluated on the mounted casts for proper diagnosis to place the implant. In case of Angle class II and III situations, the direction for mandibular fixture placement was easily evaluated from a centric relation record on articulated casts. Accordingly, the fixture was placed in angled towards the maxillary teeth or residual ridge which would help prevent prosthodontic problems when fabricating the prosthesis for proper aesthetics and function.

3.12. Diagnostic wax up

The diagnostic/study casts of each patient were duplicated using the standard duplicating procedures. The proposed implant installation site was checked on the study cast for proper alignment, direction, location, and relation to the remaining dentition. Accordingly, the future prosthesis or artificial tooth was waxed up on the duplicated cast. This diagnostic wax up helped the patient to get the idea about future tooth. When the patient was satisfied with the diagnostic wax up, it was then transferred to resin template. This resin template was used for aesthetic placement of implant and future prosthesis.

3.13. Investigations advised for each patient

Biochemical and radiographic investigations were done in order to find out any existing systemic disease or localized pathology.

Biochemical investigations were advised and evaluated:

- complete blood count (CBC)
- random blood sugar (RBS)
- fasting blood sugar (FBS)
- bleeding time and clotting time (BT and CT)
- serum creatinine (S/C)

Radiographic investigations were advised and evaluated:

- intra oral periapical (IOPA) view
- orthopantomogram (OPG)

3.14. Diagnosis

Each case was diagnosed as **Partially Edentulous Arch** with the missing of specific tooth. This definitive diagnosis was determined by the data

obtained through medical history, dental history, radiographic study, casts analysis, photograph, biochemical investigations, clinical examinations and evaluation of edentulous residual ridge space and associated structures.

3.15. Treatment Plan

A definite treatment plan of Implant Supported Restoration/Prosthesis was formulated for each patient. The findings detected through dental check up and X-ray examinations were explained to the patients in details. The available alternative treatment options were presented to each patient, and the pros and cons of each of the alternative treatment procedures was discussed and explained to the patient before determining the treatment plan of implant supported prosthesis. Every patient gave the written informed consent before going to start the surgery procedure (Appendix 2).

3.16. Implant System and Size Selection

Osstem Dental Implant (Seoul, Korea) system was used for this study. Osstem is an endosseous implant made of titanium. It consists of two parts: one Body or Fixture and one Abutment (Fig. 1). The fixture is inserted into jaw bone in first surgery and abutment is connected to the fixture in second surgery and the restoration/prosthesis is cemented or screwed on the abutment for function. This submerged type implant is characterized by an Internal Hex and 11° taper connection structure. Its body is designed with micro plus macro thread for minimizing bone resorption, optimal stress distribution, superior initial bonding stability, and facilitating placement depth adjustment.

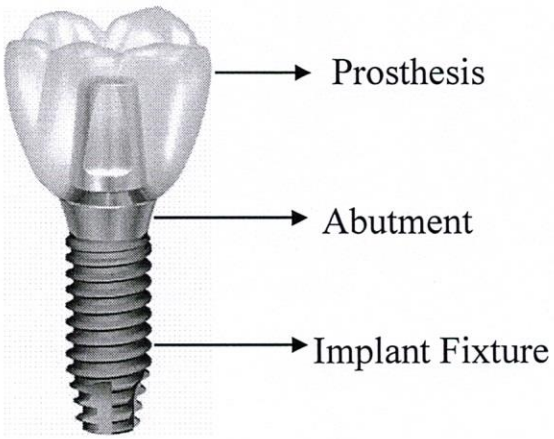


Figure 1: Dental Implant

This is a reliable system of implant that has acquired various international quality certifications (FDA, CE, ISO9001, etc.). The system has various product lines that can be optimized according to the oral cavity and surgical situation. This is the best-quality implant based on advanced developmental skills and production technologies. Osstem Implant is used in the most domestic clinical surgery cases (Osstem Surgical Manual 2006).

Length of the implant was determined by radiographic tracing and diameter was determined by bone mapping consisting with cast analysis for each patient. Implants of diameter 3.5 mm, 4 mm and 5 mm and length of 10 mm, 11.5 mm, and 13 mm were used in this study.

3.17. Sterilization Protocol

Proper sterilization protocol was maintained in every aspect, including instruments, operator, assistants, and surgical room.

3.17.1. Instruments Preparation

Titanium and stainless steel instruments were prepared separately. Titanium instruments were handled with titanium forceps only and stainless steel instruments were handled using gloved hands. They were separately washed with neutral detergents and dried and placed in two different beakers for autoclave.

3.17.2. Unit Preparation

Hand pieces and contra angles were disconnected, scrubbed with neutral detergents. They were dried and sprayed with lubricating oil to prevent internal gears from jamming and excess oil from hand piece and contra angles were wiped. The motor units were wiped with alcohol solution and set aside. The hand piece and contra angles were wrapped with surgical drapes and sterilized in an autoclave with standard method (126 degree centigrade, 15 MPa presuure, 30 minutes).

3.17.3. Room Preparation

On the day of surgery, the surgery room was cleaned and disinfected. All surfaces and washing basins, tables and machines were washed with neutral detergent, then wiped with 70% alcohol solution to disinfect and it was usually done once a day and after each surgery. Additional surfaces such as ceiling, walls, and lighting units were disinfected regularly, at least once a month. The control unit, vacuum tube, lighting unit handle, and chair were also disinfected just prior to surgery, and admittance into the room was restricted.

3.17.4. Operator and Assistant

Prior to surgery the surgical assistant changed his/her top, pants, cap, and mask. He/she performed all procedures that need to maintain the sterility

in all aspects. The operator followed the strict surgical scrub methods followed by proper gowning and gloving procedures by the help of surgical and circulating assistant.

3.18. Pre-surgical Protocol for the Patient

Every patient was counseled properly describing about the procedure of implant placement. Each patient had to undergo professional oral hygiene procedure 7 days before surgery. He/she was advised to start mouth rinsing twice a day with chlorhexidine 0.2% (Listoral, ACI Limited, Bangladesh) 2-3 days before surgery and continue for 2 weeks after surgery. Prophylaxis antibiotic with amoxicillin plus clavulanic acid (Beximco, Bangladesh) 2 gm was prescribed for each implant patient 1 hour prior to surgery. 5 to 10 mg diazepam was prescribed for the apprehensive patients on the day before surgery which helped the patient get good night sleep.

Patients were provided with collarless shirt and sterile gown, cap, and foot coverage. Females were advised not to use facial make up, and men were advised to shave facial hair. Intraoral disinfection was done by having the patient rinse with 0.1% chlorhexidine, and extra oral disinfection was done by wiping facial tissues with the same solution. Local anaesthesia (using 2% lidocaine with epinephrine 1:50,000) was done as per standard method required for the surgical sites. The patients were draped properly and sterility was maintained for surgical table, motor unit, hand piece, and suction unit.

3.19. Surgical Protocol

An ethical standard procedure was followed for surgical placement of every implant into the selected site. Every patient gave the written consent before surgery (Appendix 2).

All patients were operated under local anesthesia. Standard conditions of asepsis and sterility were strictly adhered to during the implant placement procedures. The surgical incision was made slight palatal or lingual to the crest of the edentulous ridge, with vertical releasing curvilinear incisions flaring into the vestibule in order to keep the base of the flap wider than the crestal incision width. Full-thickness subperiosteal labial and palatal/lingual flap was reflected to expose the crest and to provide visualization of the vertical cortices of bone. The incision ensured adequate buccal and lingual or palatal attached tissue on either side.

Surgical stent having guide channel was used to place the implant in the correct position with its axis parallel to the occlusal forces and the emergence of the implant angling to meet the functional cusp of the opposing teeth. Once the alveolar bone to receive the implant was exposed a flat implant bed was prepared using a 1mm straight fissure bur wherever it was found necessary. Once the implant site was prepared, a surgical guide or stent was placed intra orally and a small round bur or spiral drill was used to mark the implant site. The stent was then removed and the site was checked for appropriate facio-lingual and mesio-distal positioning. If there was an obvious crestal defect then a slight modification of the position was made. The site was then marked to a depth of 1 to 2 mm perforating the cortical bone using a small round bur. A pilot drill usually 2 mm in diameter was then drilled in marked place to establish the depth and axis of implant recipient site.

The drills were used in a reduction gear hand piece along with a physio-dispenser enabling internal as well as external irrigation to prevent excessive heat generation. In all cases the drill was used at the speed of 1200 to 1500 rpm with copious irrigation. Once the drill hole was made paralleling pin was used to check the parallelism of the drill hole to the adjacent teeth. These paralleling pin was used at each stage of surgery to ensure that the axis of the recipient site was not changed. Following the pilot drill, drills with gradually increasing diameters were used to enlarge the implant recipient site till the desired diameter corresponding to the selected implant diameter was reached. This total procedure is called osteotomy.

Having completed the procedure, the osteotom was checked for any abnormalities, if any and the selected implant was then placed into the prepared ossteotom using simple mount driver (implant inserting device) attached with hand piece followed by a hand wrench. The mucosa of surgical site was adapted for apposition and sutured with 3-0 black silk suture for each case (Appendix 20).

3.20. Platelet Rich Plasma (PRP) Preparation and Application

8 to 10 ml blood was drawn from the anticubital region of the patient with a 10 ml syringe and transferred to a container containing 1.4 ml anticoagulant (Citrate phosphate dextrose solution). It was then centrifuged for 10 minutes at 1300 rpm. The result was a separation of whole blood into a lower red blood cell region and upper straw-colored plasma region. There was relatively high concentration of platelets found in the boundary layer between these two regions. The upper straw colored platelet poor plasma layer and 1-2 mm of the top part of the RBC layer was aspirated and transferred into another container and again centrifuged

for 10 minutes at 2000 rpm. This resulted in an upper portion of clear yellow supernatant serum and the bottom red tinged layer consisting of highly concentrated platelet rich plasma. The upper clear layer was aspirated until 1.5 ml of serum was left. The contents of the tube was mixed well and transferred into a sterile container (Appendix 19). At the time of the application, the PRP was combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate). (Anila and Nandakumar 2006, Kathleen *et al.* 2010).

3.21. Implant Installation

Two types of fixture (body of implant) packages are marketed in Osstem Implant system: pre-mounted fixture package and no-mounted fixture package. In case of pre-mounted fixture package, the mount driver was connected to the fixture mount and the fixture was picked up. The fixture was positioned upward when moving to the oral cavity to avoid the dropping. The torque of engine was set according to the instruction of Osstem Implant System. 20 Ncm torque was set for mini implant with the diameter of 3.5 mm, and 30 Ncm was set for regular implant with the diameter greater than 3.5 mm. In no cases the torque was set more than 40 Ncm. After setting, the fixture was started inserting into osteotom. When the engine was stopped, the fixture mount was connected to the Mount Extension and fixture was placed to final depth using the Ratchet Wrench.

The following meticulous cares and cautions were taken during implant installation:

1. Too much torque was not applied when inserting the fixture with Ratchet Wrench.

   (If the insertion torque of 50 Ncm or more was applied, bone necrosis may have occurred, or the mount could not be separated due to too much pressure.)
2. If a squeaking noise was heard from the bone while inserting the fixture, the fixture was taken out and tried inserting again.
3. The hand piece was not used ever if it was stopped. In such cases, hand piece was removed and the Ratchet Wrench was used to place the implant to its final depth.

3.22. Post-operative Care

3.22.1. Post-operative Instructions

The following instructions were given to each patient after surgical placement of dental implant:

1. Bite down gently but firmly on the gauze packs that have been placed over the surgical areas, making sure they remain in place. Do not change them for the first hour unless the bleeding is not controlled. Do not eat, drink, spit, or talk during that time. The packs may be gently removed after one hour. (No case of this study reported persisting bleeding after removing of gauze packs and was not advised to place new packs.)

2. After leaving the clinic, take rest first day of surgery. Avoid bending, lifting and strenuous activities. Do not drive or attempt any hazardous

tasks for 24 hours of surgery. Avoid exercise for 3-5 days following surgery. Do not drive.

3. Do not disturb the surgical area today. DO NOT rinse vigorously or probe the area with any objects for 24 hours after your procedure. After 24 hours you may begin gentle rinsing with a warm saltwater solution (1/2 teaspoon salt + 8 ounces warm water).

   (Sometimes antimicrobial mouth rinses were prescribed and advised to use after 3 -4 days.)

4. It is important to keep the mouth clean. Do not brush your teeth for the first 8 hours after surgery. After this, you may brush your teeth gently, but avoid the area of surgery for 3 days. Brush and floss all other areas of your mouth in your regular fashion the day after your surgery.

5. Eat soft foods (e.g. Jao Bhat) for the first two days. Maintain a good, balanced diet. Return to normal regular meals as soon as you are able after the first two days. Drink plenty of water. Do not eat on the side of the implant surgery until the sutures are removed.

6. Avoid alcohol for 48 hours and smoking during the healing phase.

7. Apply an ice bag to the face over operated area for 15 minutes and remove for 15 minutes for the first day to minimizing the swelling

8. Continue the antibiotic therapy and chlorhexidine mouth rinses as prescribed for 7 days. Take Paracetamol (acetaminophen) 500 mg or any other pain killer (ibuprofen, Diclofenac sodium) prescribed before.

9. DO NOT try to wear your denture if told not to do so.

10. Return to the clinic after 7 days to remove the stitches.

11. Please call us or inform us if you face any difficulties without hesitation.

3.22.2. Post-operative Follow Up

The patients were monitored on a periodic basis both clinically and radiologically after 1 week, and subsequently once a month for the following 4 months. Sutures were removed 7 days after surgery. Every patient was advised to visit the Institute for evaluation of the implant, implant site, and surrounding structures. Clinical and radiographic evaluations were done at every follow up visit and the findings were recorded in prescribed data collection sheet (Appendix 3).

3.23. Evaluation of Osseointegration

3.23.1. Clinical Evaluation

Every patient was evaluated at baseline and after 1st week, 4th week, 8th week, 12th week and 16th week of surgery. Data were collected by history (Appendix 1), clinical examination and radiographic investigations on outcome variables of pain, swelling, bone resorption, imagistic value and stability at follow up visits and recorded in data collection sheet (Appendix 3). Each patient was monitored intensively to assess the pain and swelling for each day of first week. Self reported history along with visual analogue scale (VAS) for pain and verbal rating scale (VRS) for swelling was used to assess the pain and swelling for each patient (Appendix 7a and 8a). Vertical bone resorption was assessed by direct perioprobe measurement and X-ray tracing (Appendix 10a). Horizontal bone resorption was assessed by perioprobe measurement and model analysis (Appendix 11a). Imagistic value was evaluated by radiographic grading (Appendix 9). Survival criteria were identified by absence of discomfort including pain and swelling as well as absence of periimplant

radiolucency. Perimplant marginal bone loss less than 1.5 mm during the first year in function and annual bone loss thereafter not exceeding 0.2 mm were considered as the most important success criteria for implant.

3.23.2. Pain and Swelling in Terms of Discomfort

Postoperative pain and swelling were assessed after placement of implants in both study and control groups from the day 1 to till the discomfort reported by patients.

Post operative pain was evaluated by a visual analogue scale (VAS) along with specific questionnaire given to each patient (Appendix 7a). Patients were asked to answer the questionnaire and record their assessment by marking a cross on 100 mm VAS in every evening, considering the worst score of the day. The number and percentage of patients reported the pain was recorded at every day from day 1 to day 7. No patient of this study reported pain after 7^{th} day of surgery. The mean visual analogue scale score was calculated dividing the summation of VAS reported by the number of patients on each day.

Similarly, swelling was evaluated using verbal rating scale (VRS) with self reported experience by the patients (Appendix 8a). Swelling is a classical feature of acute inflammation after surgical placement of dental implant. Placement of dental implants physically insults both mucosal and alveolar tissues, causing a classical acute inflammation process that aims to eradicate damaged tissues and prepare the site for healing/osseointegration (Bryce *et al.* 2014, Arisan *et al.* 2010). Swelling reported by the percentage of patient and the intensity evaluated by VRS score was recorded from first day 8^{th} day of surgery.

3.23.3. Imagistic Evaluation by Radiograph

The imagistic changes occurred during the osseointegration process were evaluated by the post-surgery radiographies. A quantification method of these changes was adopted by grading on a scale from –3 to +3,

where:

-3 represents extended radiolucency, extended resorption

-2 medium radiolucency, medium resorption

-1 minimum radiolucency, minimum resorption

0 no change

+1 represents minimum radio-opacity, minimum osteocondensation

+2 medium radio-opacity, medium osteocondensation

+3 extended radio-opacity, extended osteocondensation

Grading was made in three regions viz., the mesial wall, the distal wall and the apical area of the neo alveola. By summed up the 3 grades, a reference value was obtained, which expressed the stage of the implant in terms of imagistic value. A positive index indicates a favorable prognostic of osseointegration, whereas a negative index indicates osseous resorption and a possible failure of the treatment.

3.23.4. Evaluation of Vertical Bone Height by Perioprobe

In the present study the direct bone measurement was carried out using perioprobe at first surgery just after implant placement and at second surgery after 16^{th} week of implant placement. All implants were submerged 1 to 3 mm below the crestal bone and distance from top of implant to the top of crestal bone was measured for each implant. The crestal bone height was measured for every implant and recorded accordingly as per data collection sheet (Appendix 3). The value obtained

by direct bone measurement was adjusted with the value obtained from X-ray tracing. If the values differed from each other, then the average of two values was considered as bone height.

3.23.5. Evaluation of Vertical Bone Height by X-ray Tracing

Height of the bone was evaluated by X-ray tracing. Two horizontal lines were drawn, one was just on crest of the alveolar bone above the top of implant and another was at the bottom of the implant. Then a vertical line was drawn along the long axis of the implant connecting the two horizontal lines. The distance of the vertical line confined by the horizontal lines was considered as height of implant and vertical bone above the implant (Appendix 10a). X-ray was taken with 100% scale at every follow up visit. The image of the implant was then placed over the 100% scale of implant measurement template. Then the height of vertical bone from the top of implant to horizontal line drawn on the top of crestal bone was determined. Thus the value was determined for each patient at 1^{st} and 2^{nd} surgery.

The value obtained by direct bone measurement was adjusted with the value obtained from X-ray tracing. If the values differed from each other, then the average of two values was considered as bone height. The adjusted values were recorded as per data collection sheet (Appendix 3). The mean value of the bone height was calculated by dividing the sum of all values by total number of implants in both groups. Unpaired 't' test was done for statistical analysis and P value of less than 0.05 was considered significant.

3.23.6. Evaluation of Horizontal bone width by Cast Analysis and Perioprobe

Bone loss was calculated by model analysis. An impression of the patient's mouth was made from which a cast was fabricated. The cast was sectioned vertically at the midpoint of edentulous space. Width of the edentulous space on the model was measured by measuring scale. The width was considered the distance between buccul surface and lingual/palatal surface at the widest point of the edentulous space just below top of the crest. The measured width on the model was bone thickness and soft tissue thickness. The tissue thickness was deducted from the total thickness to find out the exact thickness of bone at the area.

To deduct tissue thickness, an endodontic reamer was probed through the tissue at top of the crest, just above buccal/labial and lingual reflection and midway between these two of edentulous ridge in patient's mouth with proper asepsis measures and precautions. In maxillary palatal side, corresponding distance of buccal/labial side was considered as the reflection of soft tissue is absent. The depth to which the probe penetrated was measured using measuring scale and transferred on the vertically sectioned cast for each penetrated point. The points were connected by pencil and the area outside of the line was considered soft tissue. Then the exact width of the bone was measured by deducting the tissue thickness (Appendix 11a).

Just after implant placement the bucco-lingual width of crestal bone was also measured directly by means of graduated periodontal probe for each patient and the value was adjusted with value obtained from model analysis. The width was measured at the midpoint of the top of implant. If the value obtained from model analysis differed from the value obtained

by direct measurement, then the average of two values was considered as bone width.

The same preoperative bone mapping and perioprobe width measurement of crestal bone were done at second stage surgery. The values were recorded and the mean change of bone reduction was calculated for both groups of patients. Unpaired 't' test was done for statistical analysis and P value of less than 0.05 was considered significant.

3.23.7. Evaluation of Implant Stability

Stability is the first clinical criterion for successful osseointegration of implant. A mobile implant indicates failure to achieve osseointegration and is suggestive of the presence of connective tissue between the implant and the bone. An implant with greater than 0.5 mm horizontal mobility or any vertical mobility should be removed to avoid continued bone loss and future compromise of the implant site (patil *et al.* 2012).

In the present study, primary stability was evaluated at the time of implant placement and secondary stability was evaluated at the time of 2^{nd} surgery after 16^{th} week of implant placement. The periotest (PT) and implant stability quotient (ISQ) devices were used to evaluate the stability of implants (Appendix 12 And 13).

3.23.7.1. Assessment of Implant Stability with Periotest

Periotest is a dental measuring instrument used for the assessment of osseointegration of dental implants, diagnosis and assessment of periodontopathies, assessment of occlusal load and monitoring the progress of treatment of natural teeth. The periotest scale extends from –8

to + 50. The lower the value, the greater the stability/damping effect of the measured implant or tooth. The method was developed by Schutle (1988) and coworkers at the university of Tubingen and was described by d'Hoedt *et al* (1985). The underlying design principle of the periotest function is as follows, an electrically controlled rod weighing 8 g taps the implant 4 times per second at a constant speed. The rod is decelerated when it touches the implant. The greater the solidity, the higher the deceleration and thus the higher the dampening effect of the surrounding tissues. After tapping the spot recoils. Faster recoil indicates increased damping. The contact time per implant between the rod and the tooth or implant lies in the range of a millisecond and represents the real measuring parameter. In practice, the method does not use the measured contact time in millisecond as values, but it is based on the numerical scale ranging from from –8 to + 50, determined by mathematical calculation (Saini and Goyal 2012).

The values normally used in clinical practices are as follows:

PT value range	Interpretation
–8 to 0	Good osseointegration, the implant is well osseointegrated and can be loaded
1 to 9	Clinical examination is required; in most cases, the implant loading is not yet possible
0 to 50	Osseointegration is insufficient, implant must not be loaded

Periotest measurements can be taken at any stage of implantation: immediately after implantation, to measure the primary stability; at the end of the healing phase, to determine whether the osseointegration has progressed sufficiently to permit implant loading; and after prosthetic treatment for early detection of any unfavourable development (Gulden 1997).

3.23.7.2. Assessment of Implant Stability Quotient (ISQ).

The implant stability quotient (ISQ) is the value on a scale that indicates the level of stability and osseointegration in dental implants. The scale ranges from 1 to 100, with higher values indicating greater stability. The acceptable stability range lies between 55 and 85 ISQ. Implant stability quotient values are obtained using resonance frequency analysis (RFA). Resonance frequency analysis is a method used to determine the stability (the level of osseointegration) in dental implants. The stability is presented as an implant stability quotient (ISQ) value. The higher the ISQ value the higher the stability (Glauser *et al.* 2003, Sennerby and Meredith 2008).

In the present study, implant stability quotient (ISQ) was measured immediately after placement of implant as well as at the time of second surgery before prosthetic loading. The value was determined for each implant of every patient and recorded in the data collection sheet (Appendix 3).

3.23.8. Assessment of Peri-implant Tissues after Prosthetic Loading

Periodontal indices of 180 patients of the present study were evaluated for assessment of the health of peri-implant tissues by recording Plaque

Index (PI), Periodontal Probing Depth (PPD) and Bleeding on Probing (BOP) one year after prosthetic loading (Appendix 14).

The following indices were used in this study:

Plaque index (PI)

Modified Plaque Index (mPI) by Mombelli et al. (1987) was used in this study. Mombelli et al. (1987) modified the original Plaque Index (PI) introduced by Silness and Löe (1964) to assess biofilm formation in the marginal area around implants (mPI).

Score	Mombelli et al. 1987 (mPI)
0	No detection of plaque
1	Plaque can be recognized by running a probe across the smooth surface of implant
2	Plaque can be seen by naked eye
3	Abundance of soft matter

Bleeding on Probing (BOP)

Modified Gingival Index (mGI) was used to assess marginal mucosal conditions around oral implants by recording bleeding on probing. Mombelli *et al.* (1987) modified the original Gingival Index (GI) introduced by Löe (1967) to assess marginal mucosal conditions around Oral Implants.

Score	Mombelli *et al.* 1987 (mGI)
0	No bleeding when a periodontal probe is passed along the mucosal margin adjacent to the implant
1	Isolated bleeding spots visible
2	Blood forms a confluent red line on mucosal margin
3	Heavy or profuse bleeding

Peri-implant Probing Depth (PPD)

Peri-implant probing depth is calculated using periodontal probe on mesial, distal, lingual and buccal sites and then the mean probing depth is

determined for each implant. Many authors have recommended to use a plastic periodontal probe to measure the periimplant probing depth (Appendix 15), whereas 2 recent papers (Lindhe and Meyle 2008, Lang and Berglundth 2011) have suggested conventional metal periodontal probes, because they do not appear to cause any damage to either the mucosal attachment or to the implant.

In the present study, millimeter graduate metal periodontal probe was used to assess the PPD around dental implants. The value depends on the gingival height of abutment used for restoration of implant. In the present study, all abutments were selected with the gingival height ranged from 1 to 2 mm. So, the baseline periimplant probing depth was 1-2 mm.

3.23.9. Data Collection

Data were collected according to preformed structured data collection sheet at postoperative 1st, 4th, 8th, 12th and 16th week (Appendix 3). Self reporting questionnaire along with visual analogue scale (VAS) for pain and verbal rating scale (VRS) for swelling were used to collect data about postoperative pain and swelling (Appendix 7a & 8a). Along with the history implant sites were observed and examined clinically to find the swelling and to detect the soft tissue conditions and recorded accordingly. Imagistic values were calculated with radiograph at every follow up visit (Appendix 9). Vertical bone loss was measured directly with perioprobe and X-ray tracing (Appendix 10 and 10a). Horizontal bone loss was also measured directly with perioprobe and by model analysis (Appendix 11 and 11a). Stability was assessed by periotest and implant stability quotient. The vertical and horizontal bone loss and stability tests were done at 1st surgery and 2nd surgery (Appendix 12 and 13). In addition to the terms and conditions of hypothesis and objectives of the study

periimplant soft tissue conditions in terms of plaque index (PI), bleeding on probing (BOP) and periimplant probing depth (PPD) were also evaluated (Appendix 14). All these findings were recorded in data collection sheet (Appendix 3)

3.23.10. Statistical Analysis

All collected data were checked, edited and compiled on a master sheet according to the variables. Later the data were put into computer Microsoft Office Excel Worksheet for statistical analysis. Statistical analysis was done with the help of SPSS (statistical package for social science) version 22. Mean age and standard deviation was calculated for total participants as well as for male and female patients (Appendix 5). Chi-square test was done to measure significant difference between percentage of male and female patients (Appendix 5a). Unpaired 't' test was done for each outcome variable to measure the level of significance between study and control group. In the present study, the level of significance was set at 0.05 and P value <0.05 was considered statistically significant.

3.23.11. Whole Method

In this study, a total of 300 patients were treated, of them 150 patients were with dental implants using PRP (study group) and other 150 patients were treated with dental implant without PRP (control group). Till the final evaluation phase 4 patients were dropped, 2 were excluded from study group, and 5 patients were dropped and 1 patient was excluded from control group. Ultimately, a total of 288 patients, 144 from each group were evaluated in this study. The whole method have been shown in a flowchart (Fig. 2)

Flow chart showing the different stages of the research along with results, discussion, conclusion and recommendation

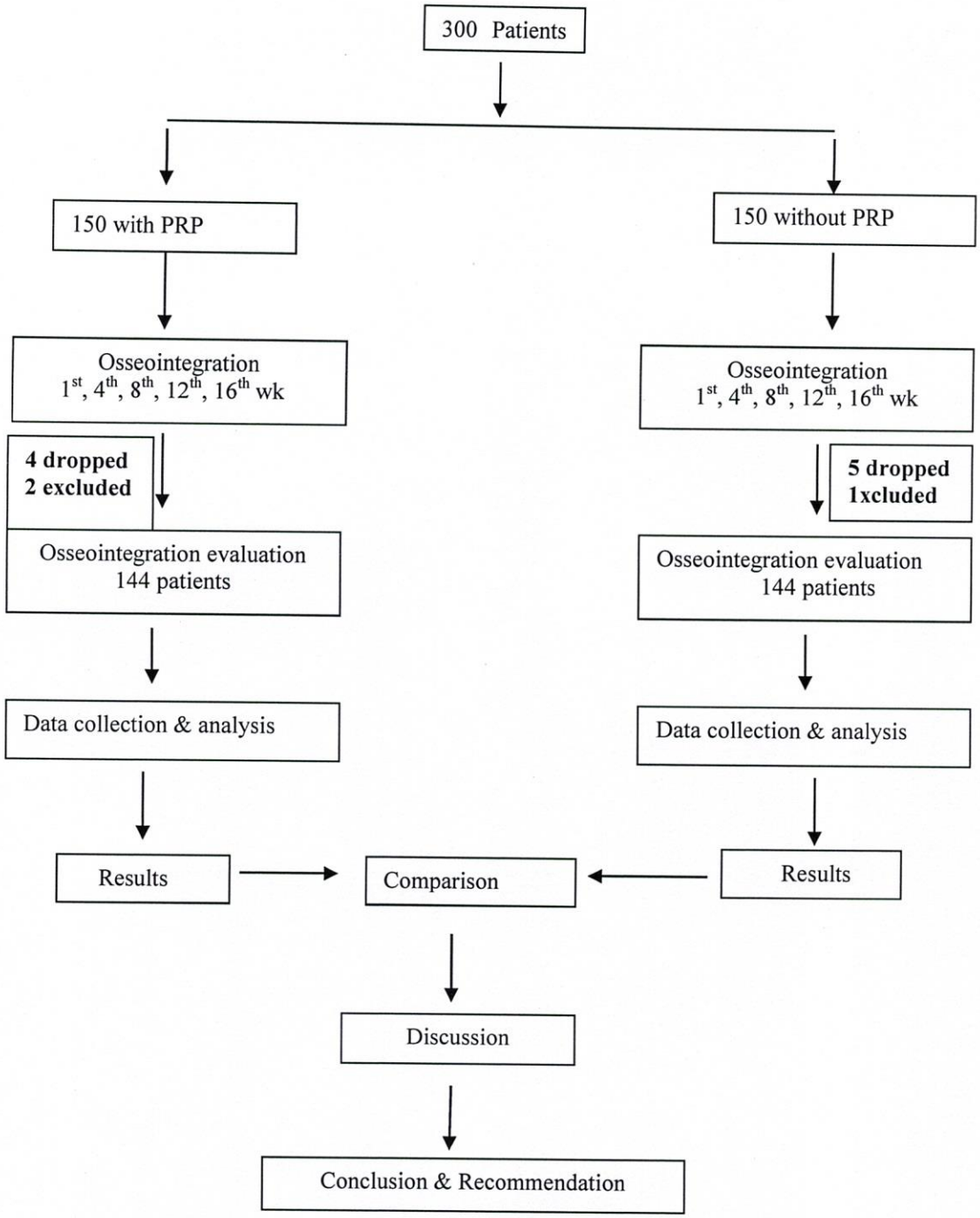


Figure 2: Flowchart showing whole method used in the study.

CHAPTER 4

RESULTS

4. RESULTS

4.1. Distribution of patients based on sex.

A total 288 patients were treated and evaluated in this study, of them 152 (52.78%) patients were males and 136 (47.22%) were females (Fig. 3). The male patients were higher in frequency and percentage than the female patients. Chi-square test was done to measure the level of significance. Difference of frequency and percentage between male and female patients was not statistically significant (Appendix 5a).

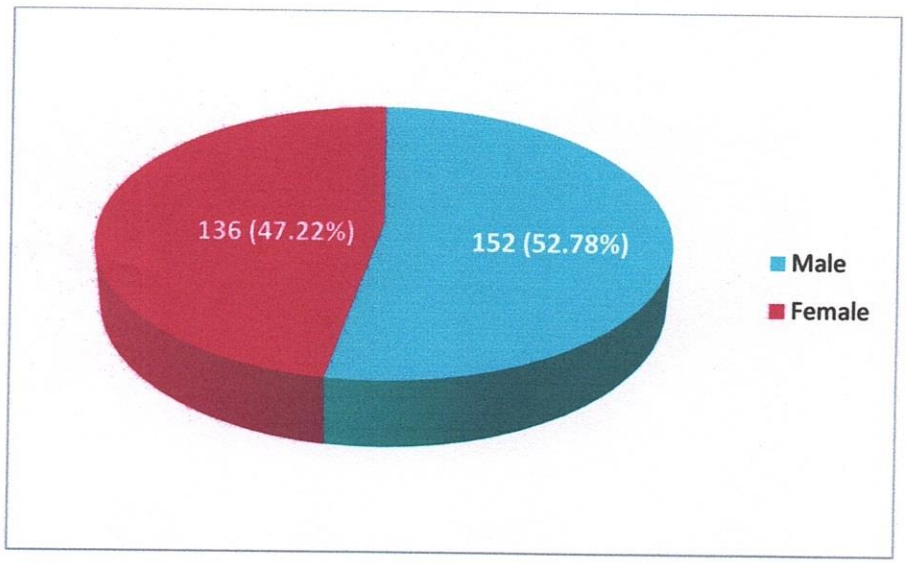


Figure 3: Distribution of patients based on sex (n=288)

4.2. Distribution of patients based on age.

The results regarding distribution of the patients based on age are shown in Figure 4 and appendix 6. The ages of the patients ranged between 22 and 82 years. The mean age of total 288 patients was 46.64 (±13.78) years. Out of 288 patients, 152 were males and their mean age was 47.21 (±14.17) years; and 136 were females and their mean age was 45.99 (±13.36) years. The highest frequencies of patients were from the age group of 43-52 years; out of 78 (27.1%) patients of this group, 40 (13.9%) were males and 38 (13.2%) were females. On the other hand, the lowest frequencies were found in the age group of 73-82 years; out of 16 (5.5%) patients of this age group, 10 (3.5%) were males and 6 (2.1%) were feamles.

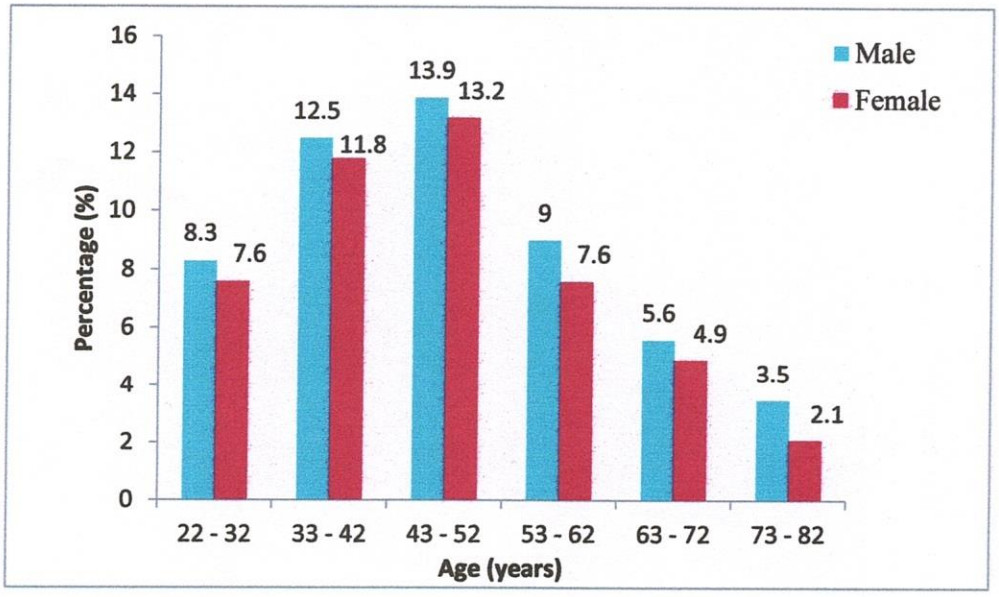


Figure 4: Distribution of patients based on age (n=288).

4.3. Distribution of implants based on study and control group.

A total 288 implants were surgically placed in 288 patients, one in each patient. Out of them, 144 (50%) implants were placed in 144 patients with plaelet rich plasma and were cosidered as study group, and other 144 (50%) implants were placed in other 144 patients without platelet rich plasma and were considered as control group (Fig. 5). The equal number of patients were selecdted in either group for comparison of effect of platelet rich plasma on osseointegration of implant.

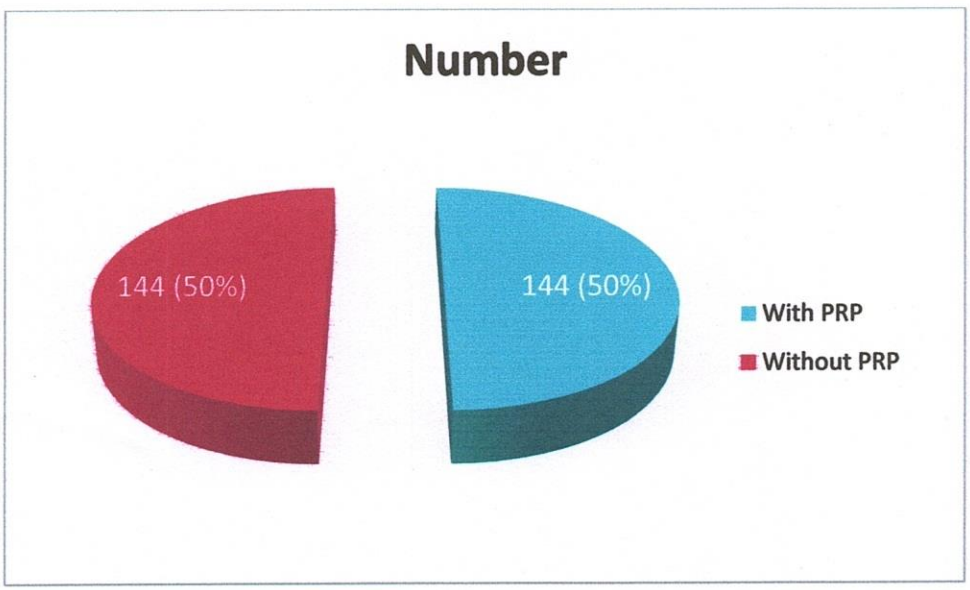


Figure 5: Distribution of implants based on study and control groups (n=288)

4.4. Evaluation of postoperative pain of the patients.

The Table 3 shows the evaluation of post operative pain after placement of implants in both study and control groups. In this study, post operative pain was evaluated by a visual analogue scale (VAS) along with specific questionnaire given to each patient. Patients were asked to answer the questionnaire and record their assessment by marking a cross on 100 mm VAS in every evening, considering the worst score of the day.

All patients of both groups reported mild pain on the first day of surgery with a peak of pain just after cessation of the effect of anaesthesia. The mean VAS score of pain was 22.67±14.19 for study group and that was 25±13.42 for the control group on the first day of surgery. On the second day of surgery, the mean VAS score was 15.72±13.92 for study group and that was 18.46±12.87 for control group. The results in the table also show that mean VAS scores were 6.00±7.95, 3.13±5.41, 1.25±2.61 and 0.42±1.31 for study group on the 3^{rd}, 4^{th}, 5^{th}, and 6^{th} day after surgery respectively. On the other hand, the scores for control group were 9.4±10.85, 5.42±8.58, 2.86±4.53, 1.75±3.93 and 0.58±2.09 on the 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day after surgery respectively.

The results reveal that the mean VAS score of pain in both groups decreased continuously from the day of surgery till the seventh postoperative day. No patient from study group reported pain after 6^{th} day but a few patients from control group reported pain on 7^{th} day of surgery. After 7^{th} post operative day no patients from either group reported any pain. The differences of mean VAS scores between the two groups on the first and second day of surgery were not statistically significant ($P>0.05$),

but the differences from the 3rd to 7th postoperative days were statistically significant (P<0.05).

Table 2: Evaluation of postoperative pain of the patients (n=288).

Reporting time	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]	df	t	p value
Day 1	22.67 ± 14.19	25.49 ± 13.42	286	1.72	0.085^{ns}
Day 2	15.72 ± 13.92	18.46 ± 12.87	286	1.73	0.084^{ns}
Day 3	6.00 ± 7.95	9.44 ± 10.85	286	3.07	0.002^{**}
Day 4	3.13 ± 5.41	5.42 ± 8.58	286	2.71	0.007^{**}
Day 5	1.25 ± 2.61	2.86 ± 4.53	286	3.69	$<0.001^{***}$
Day 6	0.42 ± 1.31	1.75 ± 3.93	286	3.85	$<0.001^{***}$
Day 7	0.00 ± 0.00	0.58 ± 2.09	286	3.35	0.001^{**}
Day 8	0.00 ± 0.00	0.00 ± 0.00			

Unpaired 't' test was done to measure the level of significance.

^{ns} Not significant (P>0.05),

** Significant (P<0.01),

*** Highly significant (P<0.001)

4.5. Evaluation of postoperative swelling of the patients.

The Table 3 and Appendix 8 show evaluation of postoperative swelling and verbal rating score of both groups of patients. A visual rating scale was used to assess the intensity of swelling. On the 1st and 2nd day of surgery 72.9% and 79.2% patients respectively from study; and 79.2% and 93.1% patients respectively from control group reported mild to moderate pain. On the first and second day the mean VRS scores were 2.31±1.05 and 2.26±0.90 respectively for study group and those were 2.51±1.04 and 2.85±0.94 for control group. On the 3rd day of surgery, the mean VRS score was 2.28±0.90 for study group and 2.82±1.11 for control group.

On the 4th, 5th, 6th and 7th day of surgery mean VRS scores were 2.14±0.82, 1.51±0.63, 1.31±0.46 and 1.14±0.35 respectively for study group; and those were 2.64±1.10, 2.32±1.13, 1.99±1.15 and 1.58±0.72 respectively for control group. On the 8th day of surgery, no patient from study group reported swelling, but a few patient from control group reported swelling and the mean VRS score of this group was 1.17±0.37.

The results reveal that swelling experience reported by the percentage of patients as well as their mean VRS score in both groups decreased continuously from the 3rd day of surgery till the 8th postoperative day; and no patient from study group reported swelling after 7th day and from control group after 8th day of surgery. The mean difference of VRS score between two groups was not statistically significant on first day of surgery, but the differences from the 2nd to 8th follow up days were statistically highly significant.

Table 3: Evaluation of postoperative swelling of the patients (n=288).

Reporting time	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]	df	t	P value
Day 1	2.31 ± 1.05	2.51 ± 1.04	286	1.57	0.117 ^{ns}
Day 2	2.26 ± 0.90	2.85 ± 0.94	286	5.44	<0.001 ***
Day 3	2.28 ± 0.90	2.82 ± 1.11	286	4.53	<0.001 ***
Day 4	2.14 ± 0.82	2.64 ± 1.10	286	4.36	<0.001 ***
Day 5	1.51 ± 0.63	2.32 ± 1.13	286	7.47	<0.001 ***
Day 6	1.31 ± 0.46	1.99 ± 1.15	286	6.65	<0.001 ***
Day 7	1.14 ± 0.35	1.58 ± 0.72	286	6.64	<0.001 ***
Day 8	1.00 ± 0.00	1.17 ± 0.37	286	5.34	<0.001 ***
Day 9	1.00 ± 0.0	1.00 ± 0.0			

Unpaired 't' test was done to measure the level of significance.

^{ns} Not significant (P>0.05)

*** Highly significant (P<0.001

VRS: verbal rating scale.

1: absence of swelling.

2: intra-oral swelling in surgical zone.

3: extra-oral swelling in surgical zone.

4: intense extra-oral swelling extended beyond surgical zone.

4.6. Evaluation of imagistic values of implants.

The Table 5 shows the mean imagistic values of implants reached at different follow up visits. In the first week the baseline mean imagistic value was -3±0.00 in both groups of implants. The mean imagistic values of study group reached -1.76±0.68 at 4th week, 0.60±1.84 at 8th week, 3.83±0.99 at 12th week and 4.94±0.95 at16th week. On the other hand, the values of control group were -2.28±0.81, -1±1.75, 1.75±1.28 and 2.65±0.61 at 4th week, 8th week, 12 week and 16th week respectably.

From the results it was observed that the implants placed with PRP showed continuous rapid progress in the terms of their imagistic values, whereas the implants placed conventionally without PRP showed stationary with slow progress in terms of imagistic values, and the mean differences were statistically highly significant.

Table 4: Evaluation of imagistic values of implants (n=288).

Evaluation period	Imagistic values of implants		df	t	P value
	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]			
1st week	-3.00 ± 0.00	-3.00 ± 0.00	286		
4th week	-1.76 ± 0.68	-2.28 ± 0.81	286	5.85	<0.001***
8th week	0.60 ± 1.84	-1.00 ± 1.75	286	7.54	<0.001***
12th week	3.83 ± 0.99	1.75 ± 1.28	286	15.43	<0.001***
16th week	4.94 ± 0.95	2.65 ± 0.61	286	24.36	<0.001***

Unpaired 't' test was done to measure the level of significance. *** Highly significant (P<0.001).

-3: extended radiolucency & resorption; -2: medium radiolucency & resorption; -1: minimum radiolucency & resorption; 0: no change; +1: minimum radio-opacity & osteocondensation; +2: medium radio-opacity & osteocondensation; +3: extended radio-opacity & osteocondensation.

4.7. Evaluation of vertical bone height around implants.

The Table 6 shows the mean marginal (vertical) bone changes at the sites of implants. The mean vertical bone height at baseline was 2.08±0.70 mm for study group and that was 2.07±0.70 mm for control group of patients. The value became 1.37±0.64 mm at 2nd surgery for study group and the mean vertical bone loss was 0.71±0.25 mm. On the other hand, the mean value was 0.86±0.35 mm at 2nd surgery for control group and the mean vertical bone loss was 1.21±0.43 mm. The study group shows the less bone loss than the control group and the difference was statistically highly significant.

Table 5: Evaluation of vertical bone height around implants (n=288).

Evaluation period with bone loss	Bone height (mm) [top of implant to top crestal bone]		df	t	P value
	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]			
1st surgery	2.08 ± 0.70	2.07 ± 0.70	286	0.08	0.933 ^{ns}
2nd surgery	1.37 ± 0.64	0.86 ± 0.35	286	8.38	<0.001 ***
Bone loss	0.71 ± 0.25	1.21 ± 0.43	286	12.04	<0.001 ***

Unpaired 't' test was done to measure the level of significance.

^{ns} Not significant (P>0.05)

*** Highly significant (P<0.001)

4.8. Evaluation of horizontal bone loss around implants.

The Table 6 shows the mean bucco-lingual (horizontal) bone loss at the sites of implants. The mean bucco-lingual bone width for study group was 6.95±1.15 mm and that was 6.93±1.14 for control group at the time of implant placement. At the time of 2nd surgery, the mean width was 6.19±0.96 mm and mean bucco-lingual bone loss was 0.76±0.43 mm for study group. On the other hand, at time of 2nd surgery the mean bucco-lingual bone width was 5.85±0.80 mm and the bone loss was 1.08±0.62 mm for control group. The difference of bone loss between two groups was statistically highly significant.

Table 6: Evaluation of horizontal bone around implants (n=288).

Evaluation period with bone los	Horizontal bone width (mm)		df	t	P value
	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]			
1st surgery	6.95 ± 1.15	6.93 ± 1.14	286	0.15	0.878 ns
2nd surgery	6.19 ± 0.96	5.85 ± 0.80	286	3.33	<0.001***
Bone loss	0.76 ± 0.43	1.08 ± 0.62	286	5.18	<0.001***

Unpaired 't' test was done to measure the level of significance

ns Not significant (P>0.05)

*** Highly significant (P<0.001)

4.9. Evaluation of implant stability by periotest.

The Table 8 shows the periotest values of both study and control groups of implants. The baseline periotest value at 1st surgery was 10.33±1.06 for study group and that was 10.29±1.05 for control group. The value became -5.29±1.10 for study group and that was -3.90±1.33 for control group after 16th week at the time of second surgery. The value changed from 1st surgery to 2nd surgery was 15.63±0.49 for study group and that changed value was 14.19±0.52 for control group. Negative value indicates stability and if greater the negative value, greater will be the stability of implants. The value became more negative in study group than that of control group at the time of 2nd surgery, which proves that the implants of study group were stable more than those of the control group. The variation was statistically highly significant.

Table 7: Evaluation of implant stability by periotest values (n=288).

Evaluation time with difference	Periotest value		df	t	P value
	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]			
1st surgery	10.33 ± 1.06	10.29 ± 1.05	286	0.33	0.738 ns
2nd surgery	-5.29 ± 1.10	-3.90 ± 1.33	286	9.65	<0.001***
Value changed	15.63 ± 0.49	14.19 ± 0.52	286	24.14	<0.001***

Unpaired 't' test was done to measure the level of significance

ns Not significant (P>0.05)

*** Highly significant (P<0.001).

4.10. Evaluation of implant stability quotient.

Table 9 shows the evaluation of implant stability quotient (ISQ) at 1st surgery just after implant placement and at 2nd surgery after 16th week of implant placement. The mean baseline ISQ at 1st surgery was 33.47±2.45 for study group and that was 33.43±2.04 for control group. After 16th week at the time of 2nd surgery, the mean ISQ was 81.74±1.22 for study group and that was 61.39±1.24 for control group. The difference of mean ISQ from 1st surgery to 2nd surgery was 48.26±1.22 for study group and that was 27.96±0.80 for control group. The difference of implant stability quotient between two groups was statistically highly significant.

Table 8: Evaluation of implant stability quotient (n=288).

Evaluation time with difference	ISQ		df	t	p value
	Study group (n_1=144) [Mean ± SD]	Control group (n_2=144) [Mean ± SD]			
1st surgery	33.47 ± 2.45	33.43 ± 2.04	286	0.15	0.876^{ns}
2nd surgery	81.74 ± 1.22	61.39 ± 1.24	286	139.48	$<0.001^{***}$
Difference	48.26 ± 1.22	27.96 ± 0.80	286	165.56	$<0.001^{***}$

Unpaired 't' test was done to measure the level of significance

^{ns} Not significant ($P > 0.05$)

*** Highly significant ($P < 0.001$).

4.11. Evaluation of periimplant indices after prosthetic loading.

The Table 10 shows the periimplant indices after 1 year of prosthetic loading. Periimplant indices were registered for each patient at each implant site. Considerations were given on plaque index (PI), bleeding on probing (BOP), peri-implant probing depth (PPD).

At the baseline the mean plaque index was 0.00 ± 0.00 for both the study and control groups. After one year of prosthetic loading the value became 0.56±0.50 for study group and that became 1.11±0.74 for control group. Similarly, at the baseline the mean bleeding on probing was 0.00 ± 0.00 for both study and control groups. After one year the value was 0.33 ± 0.47 for study group and that was 1.00 ± 0.82 for control group. The baseline periimplant probing depth was 1.56 ± 0.50 mm for study group and that was 1.44 ± 0.50 mm for control group. After one year of loading the values were 2.06 ± 0.50 mm and 2.44 ± 0.55 mm for study group and control group respectively. The differences of indices from baseline to one year were statistically very highly significant ($P < 0.001$).

Table 9: Evaluation of periimplant indices after 1 year of prosthetic loading (n=180).

Indices at evaluation time		Study group (n_1=90) (Mean ± SD)	Control group (n_2=90) (Mean ± SD)	df	t	p value
PI	Baseline	0.00 ± 0.00	0.00 ± 0.00			
	1 year	0.56 ± 0.50	1.11 ± 0.74	178	5.89	<0.001***
	Difference	0.56 ± 0.50	1.11 ± 0.74	178	5.89	<0.001***
BOP	Baseline	0.00 ± 0.00	0.00 ± 0.00	178		
	1 year	0.33 ± 0.47	1.00 ± 0.82	178	6.67	<0.001***
	Difference	0.33 ± 0.47	1.00 ± 0.82	178	6.67	<0.001***
PPD (mm)	Baseline	1.56 ± 0.50	1.44 ± 0.50	178	1.49	0.138ns
	1 year	2.06 ± 0.50	2.44 ± 0.55	178	4.95	<0.001***
	Difference	0.50 ± 0.00	1.00 ± 0.41	178	11.55	<0.001***

Unpaired 't' test was done to measure the level of significance.

ns Not significant (P > 0.05)

*** Highly significant (P < 0.001).

PI : plaque index

BOP : bleeding on probing

PPD : probing pocket depth/periodontal probing depth

CHAPTER 5

DISCUSSION

5. DISCUSSION

In this study, 288 patients were evaluated with age range between 22 and 82 years (Fg. 4 and Appendix 6). The mean age of the participants of present study was 46.64 (±13.78) years. The value for male was 47.21 (±14.17) years and for female was 45.99 (±13.36) years (Appendix 5). The highest number of patients were from the age group of 43-52 years; in case of male they were 40 (46.31%) out of 152 and in case of female they were 38 (27.94%) out of 136. On the other hand, lowest number of patients were found in the age group of 73-82 years; the male patients of this group were 10 (6.58%) and the females of this group were only 6 (4.41%).

Bertil *et al.* (1991) conducted a study comprised of 4,641 Brånemark dental implants, which were retrospectively followed from stage 1 surgery to completion of the prosthetic restorations. The implants were placed during a 3-year period (1986 to 1988) in 943 jaws, representing 889 patients with complete and partial edentulism. In their study, the mean age of the patients was 57.5 years (range 13 to 88 years) at implant placement. The mean age of the present study is not similar to that of their study because the age range of their study was from 13 to 88 years, whereas age range of the present study was between 22 and 82 years. Dissimilarity between these two studies was also found regarding the type of patients. The present study was conducted only on partially edentulous patients, but they conducted the study on both partially and completely edentulous ones.

Another similar study was conducted by Ragnar *et al.* (1990). They reviewed the long-term outcome of prostheses and fixtures (implants) in 759 totally edentulous jaws of 700 patients. Of this population, 56.8%

were females and 43.2% males. The mean age at the time of fixture placement was 55.3 years (range 19 to 79 years) which is not exactly similar to that of the present study because of their patients were totally edentulous. The present study was conducted only on the patients who were partially edentulous and the mean age was logically lower than their study on total edentulous patients.

The Fig. 3 shows the distribution of patients based on sex. In this study, total 288 patients were treated, of them152 (52.78%) were males and 136 (47.22%) were females. The male patients were higher in number than the female patients, but the difference frequency between male and female patients was not statistically significant (Appendix 5a).

Bural *et al.* (2013) conducted a study on demographic assessment including gender and age. In their demographic assessments of 616 patients 360 (58.44%) were women and 266 (43.18%) were men with a mean age of 52.12 ± 13.79 years. Their study evidenced that the females are higher in number than the males, which is reversed to the present study. This dissimilarity might be due to socioeconomic condition, religion, facilities available for women and intention of female patients to have the treatments compared to males.

Chrcanovic *et al.* (2015) conducted a meta-analysis with the aim to test the null hypothesis of no difference in the failure rates, marginal bone loss (MBL) and post-operative infection for implants inserted in male or female patients, against the alternative hypothesis of a difference. They included ninety one publications in their study with a total of 27,203 (51.96%) implants inserted in men, and 25,154 (48.04%) implants inserted in women. The results suggest that the insertion of dental implants in male patients higher than the female patients. The results of

this meta-analysis are coincided with the results of the present interventional prospective controlled clinical trial where the male and female patients were 52.78% and 47.22% respectively.

Another long term study was conducted by Ghahroudi *et al.* (2015) during 2002 to 2012 with the objective to see the frequency of dental implants placed in the esthetic zone. In their study in which total 657 implants had been placed in the maxillary esthetic zone (first premolar to first premolar). Of them, 372 (56.6%) had been placed in females and 283 (43.1%) in males. The results of mentioned study suggest that female participants were higher by percentage than male participants. So, it is revealed that the results of their study are not coincided with the present clinical trial as because in the former study the implants had been placed only in the aesthetic zone, whereas in the present clinical trial implants was placed both the aesthetic and non aesthetic zones. As females are concerned more about aesthetics, so the female patients received more implant treatments in aesthetic zone rather than male patients. Accordingly the results can be assumed logical.

The Fig. 5 shows the distribution of implants placed with and without the adjuvant therapy. A total 288 implants were evaluated in 288 patients. Of them 144 (50%) implants were placed with platelet rich plasma as an adjuvant therapy and 144 (50%) implants were placed conventionally without using any adjuvant therapy. The equal numbers of patients were selected for placement of implants with and without platelet rich plasma purposively to compare the results that were found during evaluating period.

The present study is a case control clinical trial. The case-control design is frequently used to study the discriminatory accuracy of a screening or

diagnostic biomarker. Yet, it has not been determined the appropriate ratio or number which has to be taken for cases and control for study. It is common for researchers to sample equal numbers of cases and controls, a strategy that can be optimal for studies of association (Etzioni *et al.* 1999, Pepe *et al.* 2001, Janes and Pepe2006). The present study is an interventional prospective type of clinical trial. So, it was logical to select equal number of cases and controls for this study.

The Table 2 and Appendix 7 show the evaluation of post operative pain in both study and control groups. In this study, post operative pain was evaluated by a visual analogue scale (VAS) along with specific questionnaire given to each patient. Patients were asked to answer the questionnaire and record their assessment by marking a cross on 100 mm VAS in every evening, considering the worst score of the day.

All patients of both groups reported mild pain on the first day of surgery with a peak of pain just after cessation of the effect anaesthesia. The mean VAS score of pain was 22.67±14.19 for study group and that was 25±13.42 for the control group on the first day of surgery. On the second day of surgery, the mean VAS score was 15.72±13.92 for study group and that was 18.46±12.87 for control group. The results in the table also show that mean VAS scores were 6.00±7.95, 3.13±5.41, 1.25±2.61 and 0.42±1.31 for study group on the 3rd, 4th, 5th, and 6th day respectively after surgery. On the other hand, the scores for control group were 9.4±10.85, 5.42±8.58, 2.86±4.53, 1.75±3.93 and 0.58±2.09 on the 3rd, 4th, 5th, 6th and 7th day of surgery respectively.

The results reveal that the mean VAS score of pain in both groups decreased continuously from the day of surgery till the seventh

postoperative day. No patient from study group reported pain after 6th day but a few patients from control group reported pain on 7th day of surgery, and after 7th post operative day no patient from either group reported any pain. The differences of mean VAS scores between the two groups on the first and second day of surgery were not statistically significant ($P > 0.05$), but the differences from the 3rd to 7th postoperative days were statistically significant ($P < 0.05$).

Aizenberg *et al.* (2013) conducted a pilot study with the objective to evaluate the post operative discomfort including pain and swelling. They compared findings between the implant patients treated with open flap and flapless methods. They also compared the findings between the patients received single implants and the patients received ≥ 4 implants. A numerical rating scale (NRS) was used to evaluate pain in their study. After 24 hours 1 of 5 (20%) patients in the flapless surgery group and 3 of 6 (50%) patients in the open flap surgery group reported pain. The median value of pain for the patients received single implant was maximum 7 in numerical rating scale. No single implant patient in either group reported pain at follow up visits after 3 or 7 of surgery. On the other hand, patients who received ≥ 4 implants, after 24 hours 1 of 4 (25%) patients in the flapless surgery group and 4 of 5 (80%) patients in the open flap surgery group reported pain. After 3 days of the surgery 1 of 4 (25%) patients in the flapless surgery group and 2 of 5 (40%) in the open flap surgery group reported pain up to median value of 5 in the numerical rating scale. But no patient from either group reported pain after 7 days of surgery. So, the results of the mentioned pilot study are exactly similar to those of the present clinical trial, because no patient from either study group or control group of the present study reported pain after 7 days of surgery.

Another similar study was conducted by Neto *et al.* (2014) with the aim of evaluating postoperative discomfort included pain, bleeding and swelling in single-tooth implant patients of immediate or conventional tooth restoration. In their study pain was evaluated by a questionnaire along with a visual analogue scale (VAS) given to each of the patients. Patients were asked to answer the questionnaire and recorded their assessment by marking a cross on 100 mm VAS in the every evening, considering the worst score of the day for each question. Patients from both treatment groups scored mild pain two to three hours after surgery with a peak of pain just after cessation of the analgesic effect. Pain scores decreased continuously till the third postoperative day, and no patient from either group reported mild or moderate pain after 3rd day of surgery. The decrease in pain from the first to the third postoperative day was also statistically significant.

The present clinical trial is not exactly coincided with the mentioned trial due some confounding variable like drugs taken by the patients as well as instructions given for maintenance for oral hygiene. The present study was carried out with a large sample size of 288 patients whereas their study was carried out only with 24 patients. But the difference of VAS score between the two groups of the present on 3rd day was statistically significant like the above mentioned study.

Some other studies were conducted to investigate the experience of pain and swelling after implant placement. The authors used visual analogue scale (VAS) with the related questionnaire for measuring the outcomes. Most patients reported mild to moderate pain. Average pain experience decreased significantly with time. The peak intensity of pain occurred at 24 hours of operation. The average VAS score was 24/100 on day 1,

12/100 on day 3 and 9/100 on day 6 (Hashem *et al.* 2006, Amini *et al.* 2016). These studies are consistent with the present clinical trial, but they did not record the pain after 6th day of surgery. In the present clinical trial, pain was recorded till the 7th post operative and no patient from either PRP (study) group or without PRP (control) group reported pain after 7 days of implant placement.

The Table 3 and Appendix 8 show evaluation of postoperative swelling and verbal rating score of both groups of patients. A visual rating scale was used to assess the intensity of swelling. On the 1st and 2nd day of surgery 72.9% and 79.2% patients respectively from study; and 79.2% and 93.1% patients respectively from control group reported mild to moderate pain. On the first and second day the mean VRS scores were 2.31±1.05 and 2.26±0.90 respectively for study group and those were 2.51±1.04 and 2.85±0.94 for control group. On the 3rd day of surgery, the mean VRS score was 2.28±0.90 for study group and 2.82±1.11 for control group.

On the 4th, 5th, 6th and 7th day of surgery mean VRS scores were 2.14±0.82, 1.51±0.63, 1.31±0.46 and 1.14±0.35 respectively for study group; and those were 2.64±1.10, 2.32±1.13, 1.99±1.15 and 1.58±0.72 respectively for control group. On the 8th day of surgery, no patient from study group reported swelling, but a few patient from control group reported swelling and the mean VRS score of this group was 1.17±0.37.

The results reveal that swelling experience reported by the percentage of patients as well as their mean VRS score in both groups decreased continuously from the 3rd day of surgery till the 8th postoperative day; and no patient from study group reported swelling after 7th day and from

control group after 8th day of surgery. The mean difference of VRS score between two groups was not statistically significant (P>0.05) on first day of surgery, but the differences from the 2nd to 8th follow up days were statistically highly significant (P<0.001).

Swelling is a classical feature of acute inflammation after surgical placement of dental implant. Placement of dental implants physically insults both mucosal and alveolar tissues, causing a classical acute inflammation process that aims to eradicate damaged tissues and prepare the site for healing/osseointegration (Bryce *et al.* 2014, Arisan *et al.* 2010).

A pilot study was conducted by Aizenberg *et al.* (2013) to evaluate the post operative swelling. They evaluated swelling after placement of single and multiple (≥ 4 implants) implants with flapless and open flap methods. All patients were asked to answer a questionnaire to evaluate swelling that they experienced. The swelling experiences were divided and recorded as: swelling not at all, some swelling, and a lot of swelling after 24 hours, 3 days and 7 days of surgery.

In the single implant flapless surgery group, 40% patients experienced swelling not at all, 60% experienced some swelling and no patient experienced a lot of swelling after 24 hours of surgery. No patient of this group reported any type of swelling after 3 and 7 days of surgery. In the single implant open flap surgery group all patients experienced either some swelling or a lot of swelling; of them 66.7% patients experienced some swelling and 33.3% reported a lot of swelling after 24 hours of surgery. After 3 days swelling disappeared in 16.7% patients and they reported no swelling at all but the rest 83.3% experienced some swelling

without reporting a lot of swelling by any patient. After 7 days 83.3% patients experienced swelling not all, but the remaining 16.7% patients of this group reported some swelling and no patient reported a lot of swelling.

On the other hand, in the multiple implant flapless Group, 50% patients experienced swelling not at all, 50% experienced some swelling and no patient experienced a lot of swelling after 24 hours of surgery. After 3 and 7 days 75% patients swelling experience not all but remaining 25% patients reported some swelling experience without reporting a lot of swelling by any patient.

In the multiple implant open flap surgery group all patients developed swelling; of them 20% patients experienced some swelling and 80% reported a lot of swelling after 24 hours of surgery. After 3 days 60% patients reported some swelling and 40% reported a lot of swelling. But after 7 days 100% patients of this group disappeared swelling and all patients reported swelling experience not at all.

The results of the mentioned study are similar to the results of the present clinical trial study. In the present study, 100% patients from both groups disappeared swelling after 8^{th} day surgery.

Another similar study was conducted by Neto *et al.* (2014) with the aim of evaluating postoperative discomfort in terms of swelling in single-tooth implant patients of immediate or conventional tooth restoration. Swelling was assessed by questionnaire. The form with the questions and their respective VAS was given to the patients, and they were asked to fill it in every evening, considering the worst score of the day for each

question. The visual analogue scale was graduated from 0 to 100 mm denoting the 0 as no swelling at all and 100 as immense swelling. Swelling was assessed each day after surgery for seven days. Swelling peaked on the day after the surgery and decreased up to the seventh postoperative day, but this was statistically significant only for the immediate restoration group ($P<0.05$, Friedman test). Considering the pooled data, the decrease was statistically significant ($P<0.01$, Friedman test) from the first to the third, fourth, fifth, sixth, and seventh postoperative day, showing a continuous decrease over the time period.

The results of the present study agree with the results of the study conducted by Neto *et al.* (2014). In the present study, swelling was assessed till its disappearing. In the study group, swelling peaked on the 3rd day after surgery and gradually decreased over time up to the 7th post operative day. The mean value of VRS score was 1 on the 8th post operative day which revealed that swelling disappeared in at all patients. But in the conventional control group, a few patients reported swelling till 8th post operative day and the mean value of VRS score was 1.17±03. On the 9th post operative day, the mean VRS score of control group was 1 which revealed that swelling disappeared from all patients. So the results of the present study exactly support the results of the above mentioned study.

Amini *et al.* (2016) conducted a study with the objective to compare the effects of two prophylactic oral medications in the context of post-operative management of pain and swelling following simple dental implant surgery. A total of 31 patients (11 males and 20 females, aged 26-66) were included in their study. Inflammation was recorded by verbal rating scale (VRS). In this scale, number 1 stands for the absence of

inflammation. Patients with an intra-oral swelling in the surgical zone are scored 2. Any extra-oral swelling in the surgical zone is scored 3. Number 4 signifies an intense inflammation exhibited by extra-oral swelling extended beyond the surgical zone. Regarding swelling, 54.8% of the patients in test group and 50% of the control group had no swelling on day 6 after the surgery.

The present study reveals that most of the patients disappeared swelling on the 6th post operative day with VRS score 1.31.±0.46 for study group and 1.99±1.15 for control group. Early disappearing of swelling compared to above mentioned study might be due to use of platelet rich plasma in study group, methods applied, sample size selected and the study period followed in the present study.

Another study on post operative discomfort after implant placement was conducted by Hashem *et al.* (2006). In their study 30 implants were placed in 18 patients and the post operative swelling was evaluated till the 6th post operative day. The percentage of patients reporting swelling dropped from 72% on the first day to 39% by the sixth postoperative day. The results of this study are similar to the results of the present clinical trial. In the present study, the percentage of swelling reported by the patients dropped from 72.9% on the 1st day to 30.6% on the 6th day in the test group and from 79.2% on the 1st day to 47.9% on the 6th in the control group (Appendix 8). The results of the present supposed to be similar to those of the mentioned study because the sample size was only 18 in that mentioned study, whereas sample size was 288 in the present study.

The Table 4 shows the mean imagistic values of implants reached at different follow up visits. In the first week the baseline mean imagistic value was -3±0.00 in both groups of implants. The mean imagistic values of study group reached -1.76±0.68 at 4th week, 0.60±1.84 at 8th week, 3.83±0.99 at 12th week and 4.94±0.95 at16th week. On the other hand, the values of control group were -2.28±0.81, -1±1.75, 1.75±1.28 and 2.65±0.61 at 4th week, 8th week, 12 week and 16th week respectably.

From the results it was observed that the implants placed with PRP showed continuous rapid progress in the terms of their imagistic values, whereas the implants placed conventionally without PRP showed stationary with slow progress in terms of imagistic values, and the mean differences were statistically highly significant ($P < 0.001$).

Dumitru (2011) adopted self induced imagistic indices to evaluate osseointegration of dental implants. As there is no method to describe periimplantar histological situation, conventional and digital radiographies are methods of first impression in osseointegration evaluation. In the study, 500 screw shaped implants were evaluated. The evaluation was made using classical imagistic methods that is retroalveolar and orthopantomographies. Thus, radiologically visible periimplantar tissue changes were evaluated during and after the osseointegration process.

The study was conducted to observe the imagistic changes occurred during the osseointegration process, the post surgery radiography being taken as a baseline reference. As there is no standard evaluation method of the osteoconduction and osteolysis of the periimplantar osseous tissue, the researcher conceived a self induced quantification method of these changes by grading on a scale from – 3 to + 3. Dumitru (2011) evaluated

post operative radiographic changes for 4 months. In his study, 86% implants reached mean imagistic index between +1 and +4 from, 8% reached the value over +5 during 1st to 4th month of the post operative observation period. Only 6% implants showed the negative imagistic index which were placed in D4 type bone with low density. As per observation the study showed that 80% implants was with progressive osseointegration, 7.5% was with stationery osseointegration and 12.5% implants was with the trend of retrograde osseointegration.

The present clinical trial was conducted to evaluate the effect of PRP on implant osseointegration. The study group treated with PRP showed progressive osseointegration in 100% implants and the average imagistic value reached up to 4.94±0.95 by 16th week of evaluation period. It indicates that PRP enhanced osseointegration. On the otherhand, implants in the control group treated with conventional method reached imagistic value up to 2.65±0.61 and the intermediary observational follow up at 8th and 12th week showed the stationary and slow progress in osseointegration. So, it can be concluded that the present study is consistent with the study conducted by Dumitru (2011) and PRP enhances osseointegration.

The Table 5 shows the mean marginal (vertical) bone changes at the sites of implants. The mean vertical bone height at baseline was 2.08±0.70 mm for study group and that was 2.07±0.70 mm for control group of patients. The value became 1.37±0.64 mm at 2nd surgery for study group and the mean vertical bone loss was 0.71±0.25 mm. On the other hand, the mean value was 0.86±0.35 mm at 2nd surgery for control group and the mean vertical bone loss was 1.21±0.43 mm. The study group shows the less

bone loss than the control group and the difference was statistically highly significant ($P < 0.001$).

In the present study, vertical bone height from the top of the implant to top of the crestal bone was measured directly using perioprobe and by X-ray tracing. Two values were adjusted and average value was taken when one value differed from the other. In the surgical procedure, all implants were submerged 1 to 3 mm below the crestal and the measurement was taken for each implant at first surgery just after implant placement and at second surgery after 16^{th} week of implant placement.

A similar long term clinical and radiographic study carried out by Borgonovo *et al* (2013) to evaluate the success criteria of zirconia implants. In this study they used standard periapical radiographs at the time of implant placement and 6 months, 12 months, 24 months, 36 months, and 48 months after the placements and evaluated to assess the bone resorption. The radiographic evaluation of the study indicated that mean marginal bone loss was 1.38±0.02 mm 6 months after implant insertion; 0.41±0.05 mm 6 months after prosthetic finalization except for 2 sites where it resulted as 1.5±0.06 mm. A minimal bone remodelling with a further marginal bone loss was 0.021 mm at 36 months, and 0.05 at 48 month in this radiographic evaluation irrespective of location and size of implants.

The present controlled clinical trial was conducted to evaluate the effect of PRP on implant osseointegration irrespective of location where the implant was placed, size what was chosen and even sex of the patient that was selected. In respect of radiographic evaluation both the study group of patients treated with PRP and control group treated conventionally were evaluated at the time of implant placement and 4 weeks, 8 weeks,

12 weeks, and 16 weeks after the placement and evaluated to assess the bone resorption. The standard intraoral periapical radiograph and orthopantomogram were used to evaluate the bone loss. The mean vertical bone height at baseline was 2.08±0.70 mm for study group and that was 2.07±0.70 mm for control group of patients. The value became 1.37±0.64 mm at 2nd surgery for study group and the mean vertical bone loss was 0.71±0.25 mm. On the other hand, the mean value was 0.86±0.35 mm at 2nd surgery for control group and the mean vertical bone loss was 1.21±0.43 mm. The bone loss found in study group was less than the control group and the change was statistically highly significant ($P<0.001$).

The results of the present study exactly support the study of Borgonovo *et al* (2013). The present study was evaluated only for 4 months whereas the mentioned study was evaluated for 48 months, but the first evaluation period as well as results coincided with each other. The bone loss was found to be lees in the study group of present study by 4 months remodeling time compared to that of control group of present study as well as in the patients of the mentioned study by 6 months remodeling time. This reveals that platelet rich plasma has positive effect in bone remodeling around dental implant.

According to several studies investigating criteria for implant treatment success (Albrektsson *et al*. 1986, Albrektsson and Zarb 1993), a marginal bone loss of 1.5 mm during the first year in function and an annual bone loss not exceeding 0.2 mm thereafter are considered acceptable. Br¨agger *et al* (1998) defined a radiographic criterion for implant success, a perimplant bone resorption below the limits of 0.9 to 1.6 mm during the first year in function. In the present study, the vertical bone loss was

0.71±0.25 mm in study group and that was 1.21±0.43 mm in control group after 4 months of implant placement. This vertical bone resorption was below the normal limits of 0.9 to 1.6 mm during first year in function and could be considered acceptable for success.

Padmanabhan and Gupta (2010) conducted a study to compare the crestal bone between implants placed with conventional and osteotome technique. A total of 10 units implants were placed in the maxillary anterior region of 5 patients. One implant was placed with conventional technique and another with osteotome technique and radiographic evaluation was done on the day placement and on the 180th day after placement. A statistically significant difference was found in the level of the crestal bone loss after 6 months of surgery between both groups (P=0, n=5) with less crestal bone loss in the implants of conventional technique group. The mean crestal bone loss for conventional group and osteotome group was 0.99 mm and 1.19 mm, respectively.

These above mentioned results are also coincided with the results of the present study where the mean bone loss was 1.21±0.43 mm in conventional group and that was 0.71±0.25 mm in study group treated with PRP method. Both the results are within normal range of bone resorption and can be accepted for success.

The Table 6 shows the mean bucco-lingual (horizontal) bone loss at the sites of implants. The mean bucco-lingual bone width for study group was 6.95±1.15 mm and that was 6.93±1.14 for control group at the time of implant placement. The mean width was 9.19±0.96 mm for the study group and mean bucco-lingual bone loss was 0.76±0.43 mm at the time of 2nd surgery. On the other hand, the mean bucco-lingual bone width

was 5.85±0.80 mm and the bone loss was 1.08±0.62 mm at time of 2nd surgery after 16th week. The difference of bone loss between two groups was statistically highly significant (P<0.001).

Covani *et al* (2004) conducted a study to evaluate and compare the coronal bone remodeling between immediate implants and delayed implants. In their study the mean bucco lingual distance was 10±1.52 mm for immediate implants and 8.86±2.37 mm for delayed implants at first surgery. At second-stage surgery the value was 8.1±1.33 mm and 5.8±1.26 mm in immediate and delayed implants respectively. The bone reduction was less in immediate group in their study. Though they compared the immediate implants with conventional implants, the buccolingual bone loss was more or less similar to the values found in the present clinical trial.

Cho *et al.* (2011) conducted another study to evaluate the resorption of labial bone in maxillary implants. The mean resorption of their study was 1.9±0.45 mm. The mean resorption of the present study was 0.76±0.43 mm for implants with PRP and 1.08±0.62 mm for implants without PRP. Cho *et al.* (2011) evaluated the labial bone reduction for conventionally placed implants and the results exactly supports the results found in conventional group of the present study. The results of present study also showed that the bone resorption of the study group was less than the control group. This proves that PRP played an enhancement roll on osseointegration.

Stability is the first clinical criterion for success of dental implant. According to Patil *et al.* (2012), a mobile implant indicates failure of osseointegration and the study recommended that an implant with greater

than 0.5 mm horizontal mobility or any vertical mobility should be removed to avoid continued bone loss and future compromise of the implant site. The study also showed the zero clinical mobility is the criterion for successful osseointegration of implant. The original Brånemark *et al.* (1985) protocol suggested a 3-6 months nonloading healing period to achieve adequate stability before functional loading. Digholkar *et al.* (2014) showed that osseointegration is a measure of the clinical immobility of an implant. In the present study, in addition to clinical examination implant stability was evaluated by periotest and implant stability quotient.

The Table 7 shows the periotest values of both study and control groups of implants. Two-level stability was evaluated for each implant of both groups of patients. The baseline periotest value at 1st surgery was 10.33±1.06 for study group and that was 10.29±1.05 for control group. The value became -5.29±1.10 for study group and that was -3.90±1.33 for control group after 16th week at the time of second surgery. The difference of the value from 1st surgery to 2nd surgery was 15.63±0.49 for study group and that was 14.19±0.52 for control group. Negative value indicates stability and if greater the negative value, greater will be the stability of implants. The value became more negative in study group than that of control group which proves that the implants of study group became stable more than those of the control group. The variation was statistically highly significant ($P < 0.001$).

Andruch (2014) conducted a study to assess the implant stability with periotest. In his study, thirty two implant fixtures of five different systems were evaluated during prosthetic phase after second stage surgery. The periotest value for each implant was measured. The lowest and highest

measured PTV was -8 and +8. The calculated differences between oclusally and gingivaly measured PTV values extended from 0 to 7.8. The average of differences between gingival and occlusal PTV values for maxilla and mandible was 2.95±1.6 and 3.33±2.0 respectively.

In the present study, the mean PT value at 2nd surgery for test group and control group was -5.29±1.10 and -3.90±1.33 respectively (Table 7). Periotest values were more negative on average for implants in test group than the values for implants in control group. The low mean value and more negative individual values in study group confirm the early significant osseointegration occurred with the adjunctive therapy of PRP. The reported results of the present clinical trial are consistent with the results reported by Andruch (2014) in his study.

Saini and Goyal (2012) conducted a study to evaluate the implant stability placed in freshly extracted socket using periotest device. They evaluated 10 implants palaced in 10 patients. The minimum follow up period was 6 months with the periotest readings taken at 4th, 5th and 6th month. After comparing the PT values of 4th, 5th and 6th month, it was seen that PTVs were decreasing and the reading came to negative at 6th month. They also followed up the stability even after prosthetic loading with the reading taken at 7th, 8th, 9th month and so on till 2 years, and the values showed negative in all implants which was quite significance in terms of good osseointegration. This study is consistent with the present clinical controlled trial. The present study was conducted with the large sample of 288 patients and all implants were placed at healed edentulous space, whereas the mentioned study was conducted only with 10 patients and the implants were placed immediately after extraction. In the present study the periotest values showed negative for all implants, and the PT

value was more negative in study group than control group. It also proved that PRP had positive effect on osseointegration.

Table 8 shows the implant stability quotient test values of the present study. The present study was carried to evaluate the effect of platelet rich plasma on osseointegration of dental implant. 144 Implants placed with platelet rich plasma were considered as study group and other 144 implants placed conventionally were considered as control group. Implant stability quotient (ISQ) was measured in every patient of both groups at baseline and after 16th week of implant placement. Mean ISQs recorded at baseline were 33.47±2.45 for study group and that was 33.43±2.04 for control group. After 16th week the values were 81.74±1.22 for study group and 61.39±1.24 for control group. The difference values between 1st and 2nd surgery were 48.26±1.22 for study group and that was 27.96±0.08 for control group. The differences were statistically highly significant ($P < 0.001$).

Climent et al. (2013) carried out a study on 85 implants of 23 patients with the aim to assess Ostell implant stability quotient (ISQ). With this purpose two SmartPeg transducers were used on each implant, and three measurements were registered. One measurement was registered just after implant placement and other two measurements were recorded at control appointments consecutively regardless time or location. The average register obtained with SmartPeg I in its first measurement was 72.40 ISQ ± SD 7.012, while for the second and third measurements it was 72.22 ISQ ± SD 7.318, and 72.79 ISQ ± SD 7.208, respectively. On the other hand, the average register obtained with SmartPeg II in its first measurement was 72.06 ISQ ± SD 7.070, while for the second and third measurements it was 72.59 ISQ ± SD 7.404, and 72.82 ISQ ± SD 7.010,

respectively. The values obtained in this study are reflected the values obtained in the present clinical trial.

Kanth *et al.* (2014) conducted a study on 24 patients with the aim to evaluate the implant stability determined by Osstell Mentor® resonance frequency analysis. In all patients implants were placed by one stage technique and implant stability quotients were measured at the time of implant placement, 2, 4, 8, and 12 weeks. The overall mean minimum RFA value at the time of implant placement was 66.25 + 9.6 which gradually decreased to 63.25 + 11.4 at 4 weeks and gradually increased to 68.50 + 10.2 after 3 month. The results of above mentioned study is similar to the results of control group of the present study. The present study was conducted to compare the stability quotient between implants paced with and without platelet rich plasma. On the other hand, in the above mentioned study implants were placed with one stage technique, whereas in the present study implants were placed with two stage technique and the stability quotients were measured only at the time of implant placement and at the second surgery after 4 months.

Gailani and Lateef (2015) conducted a study to evaluate the effect of platelet rich plasma on osseointegration period of dental implants. In this study, a total of 28 dental implants were inserted in edentulous maxillae or mandibles of 13 patients using a split mouth design, i.e. each patient was received at least two dental implants at the same session, one implant was implanted in association with PRP which was placed locally in one site, to serve as PRP group, and the other implant was placed without PRP, to serve as a control group. Both groups were followed with repeated implant stability measurement by means of resonance frequency

analysis at different time intervals (at the time of implant placement, 8th week, and 12th week postoperatively). The mean values of RFA at placement (primary stability) were 70.46 ± 7.32 for the control implants and 73.21 ± 8.13 for the study implants. Eight weeks postoperatively, the mean values of ISQ were 69.57 ± 10.69 at the control sites and 73.81 ± 5.90 at the PRP sites. At 12 months follow-up period, the mean values of RFA were 71.96 ± 8.51 for control implants and 74.32 ± 5.44 for PRP implants. The ISQ values of the mentioned study are similar to those the present study.

The Table 9 shows the periimplant indices 1 year after prosthetic loading. Periimplant indices were not included in the hypothesis or objective of the present study. Beyond the hypothesis and objective the study the periimplant indices of 190 implants were evaluated after one year of restoration. Periimplant indices were registered for each patient at each implant site. Considerations were given on plaque index (PI), bleeding on probing (BOP), peri-implant robing depth (PPD).

At the baseline the mean plaque index was 0.00 ± 0.00 for both the study and control groups. After one year of prosthetic loading the value became 0.56±0.50 for study group and that became 1.11±0.74 for control. Similarly, at the baseline the mean bleeding on probing was 0.00 ± 0.00 for both study and control groups. After one year the value was 0.33 ± 0.47 for study group and that was 1.00 ± 0.82 for control group. The baseline periimplant probing depth was 1.56 ± 0.50 mm for study group and that was 1.44 ± 0.50 mm for control group. After one year of loading the values were 2.06 ± 0.50 mm and 2.44 ± 0.55 mm for study group and control group respectively. The differences of indices from baseline to one year were statistically highly significant ($P < 0.001$).

Alzarea (2016) carried out a study with 92 implant patients to evaluate the periodontal health around dental implant and natural teeth after one year of prosthetic placement. In that study, the mean plaque index (PI) score was 1.24 for implants and that was 1.57 for natural teeth, the mean bleeding on probing (BOP) was 25.96 for implants and 25.42 for natural teeth and the mean periodontal probing depth (PPD) was 2.9 for implants and that was 2.78 for natural teeth. The results of the study (Alzarea 2016) are similar to the results of the present study except mean value of bleeding on probing. This disparity might be due to variation of sample size and the oral hygiene practiced by patients between two studies.

Another similar study on 10 implants was conducted by Rajpal *et al* (2014) to evaluate the periodontal tissue around loaded implants. They assessed the PI, BOP and PPD at baseline, after 1, 3 and 6 moths of prosthetic loading. In their study the mean plaque index (PI) was 0.48 ± 0.14 at baseline and that was 0.30±0.06 after 6 months, the mean bleeding on probing (BOP) was 0.68±0.12 and 0.45±0.10 at baseline and after 6 months respectively, and the mean peri-implant probing depth (PPD) was 1.85±0.20 and 2.33±0.21 at baseline and after 6 months respectively. The periimplant indices values of the present study were more or less similar to the values found in the study conducted by Rajpal Rajpal *et al* (2014).

Borgonova *et al.* (2013) carried out a study to evaluate the success criteria 35 single piece zirconia dental implants. In their study, periodontal indices in terms of plaque index, bleeding on probing and peri-implant probing depth were registered every 6 months after final prosthetic rehabilitation. The mean PI, BOP and PPD were 1, 1 and 3

respectively after 6 months; and those were 0.5, 0.25 and 2.98 respectively after 1 year of prosthetic rehabilitation. The results of this study are consistent with the result of the present clinical trial.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6. CONCLUSION AND RECOMMEDATION

6.1. CONCLUSION

From the observation of this study it can be concluded that the use of platelet rich plasma in implant surgery increases the rate of bone formation around the implant and reduces healing time. The placement of implants with PRP reduces pain, bleeding, swelling and inflammation. PRP enhances the soft tissue repair and wound healing. Use of PRP in implant surgery reduces vertical and horizontal bone resorption of residual alveolar ridge. The use of this autologous product eliminates concerns about immunogenic reactions and disease transmission. PRP allows early implant loading. Ultimately, PRP provides an advantageous environment for acceptance of dental implants to enhance the early osseointegration.

6.2. RECOMMENDATION

Based on the outcomes of the research work the following features can be recommended:

1. Platelet rich plasma (PRP) may become a routine adjuvant therapy in addition to standard meticulous implant surgery.
2. Platelet rich plasma can be used as a medium for surface treatment of implant at the time of its placement.
3. In case of bone grafting PRP can also be used as a medium to form a sticky gel for its easy application to the surgical site.
4. PRP can be used as an adjunct therapy during the implant placement in weak bone.
5. PRP may be used to stimulate bone intrinsic factors for implant osseointegration.

6. PRP can be used in implant surgery to provide an advantageous environment for acceptance of dental implants, to eliminate immunogenic reactions and to prevent disease transmission.
3. It is also recommended to design further well controlled randomized clinical studies to assess and confirm the efficacy of clinical use of PRP.

6.3. LIMITATION OF THE STUDY

Limitations of the study included:

1. The study was conducted with a short follow up only of 16 weeks up to prosthetic loading. Post prosthetic follow up should be included in further research.
2. Stability of implants was measured with clinical mobility test, periotest value and implant stability quotient, but other more sensitive devices could not be used to measure the stability.
3. Bone height and width were measured directly with perioprobe and one dimensional radiographic tracing, the three dimensional CBCT and other digital imaging techniques were not used in this study.
4. The effect of PRP was assessed in general, but the study did not show the prognosis and effect in different type bone qualities.
5. The study was carried out on different sizes of implants, same length and diameter of implants may have shown more accurate results.
6. Surface topography and characteristics were not considered in this study which might have influenced osseointegration.
7. Histological analysis is gold standard to assess the bone density formed after any intervention. The present study was a clinical trial with human population, and this is because it was not possible to assess bone density with histology.

CHAPTER 7

SUMMARY

7. SUMMARY

This interventional controlled clinical trial entitled Effect of Platelet Rich Plasma on Osseointegration of Dental Implant was carried out under the Institute of Biological Sciences (IBSc) of University of Rajshahi from August 2012 to December 2016.

Its background study showed that dental implant can be the substitute of tooth root analogue and is surgically placed into jaw bone to support and retain the artificial tooth. It offers many advantages over conventional fixed or removable treatment options and in many cases is the treatment of choice. The clinical success of implant therapy in edentulous and partially edentulous patients is well documented and clinicians realize the benefits of adopting implant therapy in their practices. Presently, it is an integral part of mainstream dentistry, and a highly predictable and unique treatment modality to replace missing teeth. The long-term clinical success of dental implant is related to its early and optimal osseointegration. Despite the ongoing improvement in implant characteristics, bone intrinsic potential for osseointegration may be stimulated with adjuvant therapies to standard surgical procedures to achieve the best possible implant osseointegration into the adjacent bone and to ensure its long term success.

In the present study, autologous platelet rich plasma (PRP) was used as an adjuvant therapy to standard surgical procedure of dental implant. PRP reduces of post operative complication, stimulates wound healing and enhances bone regeneration around dental implant providing the most advantageous environment for its acceptance. It is safe due to its autologous nature and free from risk of cross reactivity, immune reaction or disease transmission, and it can be produced as needed from patient's

own blood. So, the general objective of this study was the evaluation of the effect of platelet rich plasma on osseointegration of dental implant with the hypothesis that it enhances osseointegration.

Total 288 implant sites from 300 consecutively selected and treated with single implant were evaluated in this study. Before implant placement each patient was selected by a thorough medical and dental history, meticulous clinical examination, radiological evaluation, and biochemical investigation supporting the specific exclusion and inclusion criteria. Of the 288 implants, 144 were placed with platelet rich plasma and considered them as study group, and other 144 implants were placed without platelet rich plasma and considered as control group.

Standard pre-surgical sterilization protocol was maintained for every patient and an ethical standard procedure was followed for surgical placement of every implant into selected site. All patients were previously informed about implants system, its merits and demerits and possible alternative treatment options. Every patient gave the written consent before surgery. Under prophylaxis antibiotic and local anaesthesia horizontal off crestal with required vertical releasing incisions were given and full thickness subperiosteal flap was reflected. Surgical stent having guide channel was used to place the implant in correct position and angulation. Osteotomy was done using the sequential diameter of drills attached in a reduction gear hand piece along with a physio-dispenser having internal as well as external irrigation system to prevent excessive heat generation. In all cases the drill was used at the speed of 1200 to 1500 rpm with copious irrigation. In the mean time PRP was prepared from the patient's own blood drawn before starting the surgery.

A table top centrifugal machine was used to prepare the PRP. 8 to 10 ml blood was drawn from the anticubital region of the patient with a 10 ml syringe and transferred to a container containing 1.4 ml anticoagulant (Citrate phosphate dextrose solution). It was then centrifuged for 10 minutes at 1300 rpm. The result was a separation of whole blood into a lower red blood cell region and upper straw-colored plasma region. There was relatively high concentration of platelets found in the boundary layer between these two regions. The upper straw colored platelet poor plasma layer and 1-2 mm of the top part of the RBC layer was aspirated and transferred into another container and again centrifuged for 10 minutes at 2000 rpm. This resulted in an upper portion of clear yellow supernatant serum and the bottom red tinged layer consisting of highly concentrated platelet rich plasma. The upper clear layer was aspirated until 1.5 ml of serum was left. The contents of the tube was mixed well and transferred into a sterile container. At the time of the application, the PRP was combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate).

The prepared osteotomy was checked and evaluated for its perfection and selected implant was placed maintaining all asepsis measures with meticulous precautions. The prepared PRP was placed at the surgical site all around the implant. Suturing was done and the instructions were given for maintenance of the implant as well as his/her oral health. Patients were advised to come for recall visits after 1^{st}, 4^{th}, 8^{th}, 12^{th}, and 16^{th} week for evaluation implant sites. Each patient was monitored intensively to assess the pain and swelling for each day of first week. Data were collected by history, clinical and radiographic investigations on outcome variables of pain, swelling, bone resorption, imagistic value and stability

at every follow up visit. Collected data were edited and calculated for presentation as results. Unpaired 't' test was done and P value < 0.05 was considered the result as statistically significant.

Results showed that a total 288 implant sites of 288 patients were evaluated in this study. Of them 152 (52.78%) patients were males and 136 (47.22%) were females. The ages of the patients ranged between 22 and 82 years with the mean age 46.64 (±13.78) years. Out of the 288 implants, 144 (50%) were placed with PRP and cosidered as study group, and other 144 (50%) were placed without platelet rich plasma and considered as control group.

Postoperative discomfort of the patients were evaluated in terms of pain and swelling. Postoperative pain was evaluated by a visual analogue scale (VAS). All patients reported mild pain on the first day of surgery and the mean VAS score was 22.67±14.19 for study group and that was 25±13.42 for the control group. The mean VAS scores also were 15.72±13.92, 6.00±7.95, 3.13±5.41, 1.25±2.61 and 0.42±1.31 for study group on the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, and 6^{th} day of surgery respectively; and the values for control group were 18.46±12.87, 9.4±10.85, 5.42±8.58, 2.86±4.53, 1.75±3.93 and 0.58±2.09 on the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day of surgery respectively.

Postoperative swelling was evaluated by verbal rating scale (VRS). The VRS scores were 2.31±1.05, 2.26±0.90, 2.28±0.90, 2.14±0.82, 1.51±0.63, 1.31±0.46 and 1.14±0.35 for study group on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day of surgery respectively. The values for the control group were 2.51±1.04, 2.85±0.94, 2.82±1.11, 2.64±1.10, 2.32±1.13, 1.99±1.15, 1.58±0.72 and 1.17±0.37 on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} day of surgery respectively. No patient of study group reported swelling after 7^{th}

day, but a few patients of control group reported pain till 8th postoperative day.

The mean imagistic values were rated on 1st, 4th, 8th, 12th and 16th week of implant placement. In the first week the baseline mean imagistic value was -3±0.00 in both groups of implants. The mean imagistic value of study group reached -1.76±0.68 at 4th week, 0.60±1.84 at 8th week, 3.83±0.99 at 12th week and 4.94±0.95 at16th week. On the other hand, the values of control group were -2.28±0.81, -1±1.75, 1.75±1.28 and 2.65±0.61 at 4th week, 8th week, 12 week and 16th week respectably.

The mean marginal (vertical) bone loss was assessed at baseline and 2nd surgery. The mean vertical bone height was 2.08±0.70 mm and 1.37±0.64 mm at baseline and 2nd surgery respectively, and the bone loss was 0.71±0.25 mm for study group. For the control group, the values were 2.07±0.70 mm and 0.86±0.35 mm at baseline and 2nd surgery respectively, and vertical bone loss was 1.21±0.43 mm.

The bucco-lingual (horizontal) bone loss at the sites of implants was measured. The mean bucco-lingual bone width for study group was 6.95±1.15 mm and that was 6.93±1.14 for control group at the time of implant placement. At the time of 2nd surgery, the mean width was 6.19±0.96 mm and mean bucco-lingual bone loss was 0.76±0.43 mm for study group. On the other hand, at time of 2nd surgery the mean bucco-lingual bone width was 5.85±0.80 mm and the bone loss was 1.08±0.62 mm for control group.

Clinical implant stability was evaluated by periotest and implant stability quotient. The baseline periotest value at 1st surgery was 10.33±1.06 for

study group and that was 10.29±1.05 for control group. The value became -5.29±1.10 for study group and that was -3.90±1.33 for control group after 16th week at the time of second surgery. The value changed from 1st surgery to 2nd surgery was -15.63±0.49 for study group and that changed value was -14.19±0.52 for control group. Negative value indicates stability and if greater the negative value, greater will be the stability of implants.

The baseline ISQ at 1st surgery was 33.47±2.45 for study group and that was 33.43±2.04 for control group. After 16th week at the time of 2nd surgery ISQ was 81.74±1.22 for study group and that was 61.39±1.24 for control group. The difference of ISQ from 1st surgery to 2nd surgery was 48.26±1.22 for study group and that was 27.96±0.80 for control group.

In addition to hypothesis testing of the present study, periimplant indices of the of 180 implants were assessed after one year of prosthetic loading At the baseline the mean plaque index was 0.00 ± 0.00 for both the study and control groups. After one year of prosthetic loading the value became 0.56±0.50 for study group and that became 1.11±0.74 for control. Similarly, at the baseline the mean bleeding on probing was 0.00 ± 0.00 for both study and control groups. After one year the value was 0.33 ± 0.47 for study group and that was 1.00 ± 0.82 for control group. The baseline periimplant probing depth was 1.56 ± 0.50 mm for study group and that was 1.44 ± 0.50 mm for control group. After one year of loading the values were 2.06 ± 0.50 mm and 2.44 ± 0.55 mm for study group and control group respectively. The differences of indices from baseline to one year were statistically highly significant ($P < 0.001$).

Based on the results of this study it can be concluded that the use of platelet rich plasma in implant surgery helps soft tissue repair, reduces pain and swelling, reduces healing time, and increases the rate of bone formation around the implant. So, it is proved and accepted the alternative hypothesis of this study rejecting the null one.

From the review of this study it can be recommended that platelet rich plasma might become a routine adjuvant therapy in addition to standard surgical procedure for implant placement to enhance the osseointegration.

There were some limitations during this research work. The bone density could not be assessed by three dimensional imaging techniques due to non availability in the country. Stability test was done with periotest device and implant stability quotient value, but other more sensitive devices could not be used to measure the implant stability.

REFERENCES

REFRENCES

Adell R, Eriksson B, Lekholm U, Brånemark PI and Jemt T (1990). A long term follow up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Impl.* 5: 347-359.

Aimetti M, Romano F, Dellavia C and De Paoli S (2008). Sinus grafting using autogenous bone and platelet-rich plasma: histologic outcomes in humans. *The International Journal of Periodontics & Restorative Dentistry.* 28(6): 585-91.

Aizenberg A, Jansson J and Abrahamsson P (2013). Short-term postoperative discomfort in patients receiving flapless contra open implant surgery. *Open Journal of Stomatology.* 3:298-305.

Alberius P (1983). Bone reactions to tantalum markers. A scanning electron microscopic study. *Acta Anat (Basel).* 115:310-318.

Albrektsson T (1983). Direct bone anchorage of dental implants. *J Prosthetic Dent.* 50: 255-261.

Albrektsson T and Hansson HA (1996). An ultrastructural characterization of the interface between bone and sputtered titanium or stainless steel surfaces. *Biomaterials.* 7:201-205.

Albrektsson T and Wennerberg A (2004). Oral implant surfaces: Part 2-review focusing on clinical knowledge of different surfaces. *Int J Prosthodont.* 17:544-564.

Albrektsson T and Zarb GA (1989). *The Branemark osseointegrated implant. Chicago: Quintessence Pub. Co. ISBN* 978-0-86715-208-1

Albrektsson T and Zarb GA (1989). The Branemark osseointegrated implant. Chicago: Quintessence Pub. Co.

Albrektsson T and Zarb GA (1993). Current interpretations of the osseointegrated response: clinical significance. *The International Journal of Prosthodontics.* 6(2): 95-105.

Albrektsson T, Branemark PI, Hansson HA and Lindstrom J (1981). Osseointegrated titanium implants. Requirements for ensuring a long-lasting direct bone-to-implant anchorage in man. *Accta Orthop Scand.* 2:155-170.

Albrektsson T, Hansson H, Kasemo B, Larsson K, Lundstrom I, McQueen D and Skalak R (1983). The interface of inorganic implants *in vivo*; titanium implants in bone. *Annals of Biomedical Engineering.*11:1-27.

Albrektsson T, Zarb G, Worthington P, and Eriksson AR (1986). The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *The International Journal of Oral & Maxillofacial Implants*. 1(1):11-25.

Alzarea BK (2016). Assessment and Evaluation of Quality of Life (OHRQoL) of Patients with Dental Implants Using the Oral Health Impact Profile (OHIP-14) - A Clinical Study. *J Clin Diagn Research*. 10(4): 57-60.

Amany AE, Hadir FE and Sameh TM (2006). Effect of PRP on osseointegration of endosseous implants in induced diabetetic experimental model. *Clinical Oral Implant Res*. 52(42): 23

Amini F, Shayesteh YS and Alikhasi M (2016). Preemptive Ibuprofen and Orally Administered Dexamethasone for Prevention of Pain and Swelling following Implant Surgery. *Regeneration, Reconstruction & Restoration.* 1(2):94-99.

Anand U and Mehta DS (2012). Evaluation of immediately loaded dental implants bioactivated with platelet-rich plasma placed in the mandibular posterior region: a clinico-radiographic study. *J Indian Soc Periodontol.* 16:89-95.

Andrewleon SQ (2011). *Consequences of tooth loss on oral function and need for replacement of missing teeth among patients attending Muhimbili Dental Clinic*, A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Dentistry (Restorative Dentistry) of Muhimbili University of Health and Allied Sciences, Tanzania. Pages: 63

Andruch K (2014). The clinical significance of two-level PVT Periotest® measurements-in vivo endosteal implant stability study. *Dental Forum.* 42*(*1):19-25.

Anila S and Nandakumar K (2006). Applications of Platelet Rich Plasma for Regenerative Therapy in Periodontics. *Trends Biomater. Artif. Organs*. 20(1):78-83.

Anitua E (1999). Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants.* 14:529-535.

Anitua E, Andia I, Ardanza B, Nurden P and Nurden AT (2004). Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost.* 91: 4-15.

Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G and Andia I (2006). New insights into and novel applications for platelet rich plasma fibrin therapy. *Trends Biotechnol.* 24(5):227-234.

Anitua E, Sánchez M, Orive G and Andía I (2007). The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Int. Journal of Biomaterials*. 28: 4551-4560.

Anitua EA (2006). Enhancement of osseointegration by generating a dynamic implant surface. *J Oral Implantol.* 32:72-76.

Anselme K (2000). Osteoblast adhesion on biomaterials. *Biomaterials.* 21:667-681.

Antonino A, Maria EL, Bianca P and Giuseppina C (2013). Platelet-rich plasma (PRP) in dental and oral surgery: from the wound healing to bone regeneration. *Immun Ageing*. 10: 23.

Arisan V, Karabuda CZ and Ozdemir T (2010). Implant surgery using bone-and mucosa-supported stereolithographic guides in totally edentulous jaws: surgical and post-operative outcomes of computer-aided *vs*. standard techniques. *Clin Oral Implants Res* 2010; 21: 980–988.

Arthur BNJr., Sargio LSdeS, Raquel RMde B, Karnia KYP, Giovanna I and Adriano P (2010). Influence of implant surfaces on osseointegration. *Braz Dent J.* 21(6):471-481.

AutoloGel System (2009). Cytomedix*: Regenerative Biotherapies.* Available at http://www.cytomedix.com/autologel/clinicalevidence .html, accessed on 20. 10. 2010.

Bertil Friberg, Torsten Jemt and Ulf Lekholm (1991). Early Failures in 4,641 Consecutively Placed Brånemark Dental Implants: A Study From Stage 1 Surgery to the Connection of Completed Prostheses. *Int. J Oral Mxillofac Implants*. 6:142-146.

Bhanot S and Alex JC (2002). Current applications of platelet gels in facial plastic surgery. *Facial Plast Surg.* 18(1):27-33.

Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Krol W and Wielkoszynski T (2007). Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: an in vitro study. *J Bone Joint Surg Br.* 89(3): 417-420.

Bobyn JD, Pilliar RM, Cameron HU and Weatherly GC (1980). The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. *Clin Orthop Relat Res*. 150:263-270.

Borgonovo AE, Censi R, Vavassori V, Dolci M, Guirado JLC, Ruiz RAD, and Maiorana C (2013). Evaluation of the Success Criteria for Zirconia Dental Implants: A Four-Year Clinical and Radiological Study. *Int J Dent*. :1-7.

Borsari V, Giavaresi G, Fini M, Torricelli P, Tschon M, Chiesa R, Chiusoli L, Salito A, Volpert A and Giardino R (2005). Comparative *in vitro* study on a ultra-high roughness and dense titanium coating. *Biomaterials*. 26:4948-4955.

Bortoluzzi MC, Traert J, Lasta R, Da Rosa TN, Capella DL and Presta AA (2012). Tooth loss, chewing ability and quality of life. *Contemporary Clinical Dentistry*. 3(4):393-397.

Boyan BD, Bonewald LF, Paschalis EP, Lohmann CH, Rosser J, Cochran DL, Dean DD, Schwartz Z and Boskey AL (2002). Osteoblast-mediated mineral deposition in culture is dependent on surface microtopography. *Calcif Tissue Int*. 71:519-529.

Br¨agger U, H¨afeli U, Huber B, H¨ammerle CFH, and Lang NP (1998). Evaluation of postsurgical crestal bone levels adjacent to non-submerged dental implants. *Clinical Oral Implants Research*. 9(4): 218-224.

Brånemark PI (1983). Osseointegration and its experimental background. *The Journal of Prosthetic Dentistry*. 50(3): 399-410.

Branemark PI (2003). *Close to the Edge - Brånemark and the Development of Osseointegration.* (Ed. Elaine McClarence).

Branemark PI, Breine U, Adell R, Hansson BO, Lindstrom J and Ohlsson A (1969). Intraosseous anchorage of dental prostheses. 1. Experimental studies. *Scand J Plast Reconstr Surg*. 3:81-93.

Brånemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O and Ohman A (1977). Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl*. 16:1-132.

Branemark PI, Zarb G and Albrektsson T (1985). *Tissue-Integrated Prosthese, Osseointegration in Clinical Dentistry*. Chicago, Quintessence Publishing Co. Inc.: 11-76.

Brånemark PI, Zarb GA and Albrektsson T (1985). Tissue-integrated prostheses: osseointegration in clinical dentistry. *Chicago: Quintessence.* ISBN 978-0-86715-129-9

Breme J, Steinhauser E and Paulus G (1988). Commercially pure titanium Steinhauser plate-screw system for maxillofacial surgery. *Biomaterials*. 9:310-313.

Browne M and Gregson PJ (2000). Effect of mechanical surface pretreatment on metal ion release. *Biomaterials*. 21:385-392.

Brune D, Evje D and Melsom S (1982). Corrosion of gold alloys and titanium in artificial saliva. *Scand J Dent Res*; 90:168-171.

Bryce G, Bomfim DI, and Bassi GS (2014). Pre- and post-operative management of dental implant placement. Part 2: management of early-presenting complications. *British Dental Journal*. 217(4): 171-176.

Bural C, Bilhan H, Cilingir A and Geçkili O (2013). Assessment of demographic and clinical data related to dental implants in a group of Turkish patients treated at a university clinic. *J Adv Prosthodont*. 5(3):351-358.

Cabbar F, Güler N, Kürkcü M, Işeri U and Sençift K (2011). The effect of bovine bone graft with or without platelet-rich plasma on maxillary sinus floor augmentation. *J Oral Maxillofac Surg*. 69:2537-2547.

Carlson NE and Roach RB Jr. (2002). Platelet-rich plasma: clinical applications in dentistry. *J Am Dent Assoc*. 133:1383-1386.

Carlsson L, Rostlund T, Albrektsson B, albrektsson T and Branemark PI (1986). Osseointegration of titanium implants. *Acta Orthop Scand.* 57:285-289.

Chappard D, Aguado E, Hure G, Grizon F and Basle MF (1999). The early remodeling phases around titanium implants: a histomorphometric assessment of bone quality in a 3- and 6-month study in sheep. *Int J Oral Maxillofac Implants*. 14:189-196.

Cho YB, Moon SJ, Chung CH and Kimm HJ (2011). Resorption of labial bone in maxillary anterior implant. *J Adv Prosthodont*. 3(2):85-89.

Chrcanovic BR, Albrektsson T and Wennerberg A (2015). Dental implants inserted in male versus female patients: a systematic review and meta-analysis. *J Oral Rehabil*. 42(9):709-722.

Climent MH, García RS, Santos RJ, Ruiz MMR, Palacin AF, Calvo PL, Bullón P and Santos JVR (2013). Assessment of Osstell ISQ's reliability for implant stability measurement: A cross-sectional clinical study. *Med Oral Patol Oral Cir Bucal*. 18(6):e877-e882.

Cochran DL, Nummikoski PV, Higginbottom FL, Hermann JS, Makins SR and Buser D (1996). Evaluation of an endosseous titanium implant with a sandblasted and acid-etched surface in the canine mandible: radiographic results. *Clin Oral implants Res*. 7:240-252.

Covani U, Bortolaia C, Barone A and Sbordone L (2004). Bucco-Lingual Crestal Bone Changes After Immediate and Delayed Implant Placement. *Journal of Periodontology*. 75(12):1605-1612.

Craddol HL and Youngson CC (2004). A study of the incidence of overeruption and occlusal interference in unopposed posterior teeth. *Br Dent J*. 196(6):341-348

Creugers NHJ and Hof Vant MA (1991). An Analysis of Clinical Studies on Resin-bonded Bridges. *Journal of Dental Research (JDR).* 70(2): 146-149

d'Hoedt, Schutle WQ (1985). The periotest research and clinical trials. *Dtsch Zahnarztl.* 40: 113-135

Daif ET (2012). Effect of autologous platelet-rich plasma on bone regeneration in mandibular fractures. *Dent Traumatol.* 29(5):399-403.

Davies J (1998). Mechanisms of Endosseous Integration. *International Journal of Prosthodontics.* 11:391-401.

de Vicente JC, Recio O, Martin-Villa L, Junquera LM and Lopez-Arranz JS (2006). Histomorphometric evaluation of guided bone regeneration around implants with SLA surface: an experimental study in beagle dogs. *Int J Oral Maxillofac Surg.* 35:1047-1053.

Digholkar S, Madhav VNV, Palaskar J (2014). Methods to Measure Stability of Dental Implants. *Journal of Dental and Allied Sciences.* 3 (1):17-23.

Dimitriou R and Babis GC (2007). Biomaterial osseointegration enhancement with biophysical Stimulation. *J musculosketel Neuronal Interact.* 7(3):253-263

Driver VR, Hanft J, Fylling CP and Beriou JM (2006). A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. *Ostomy Wound Manage.* 52(6): 68-70.

Dumitru GS (2011). *Imagistic evaluation of dental implant osseointegration.* " Lucian Blaga" University of Sibiu "Victor Paplian" Faculty of Medicine. PhD Thesis, SIBIU 2011.

El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M and Van DT (2007). Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. *J Periodontol.* 78: 661-669.

Etzioni R, Pepe M, Logton G, Hu C and Goodman G (1999). Incorporating the time dimension in receiver operating characteristic curves: a case study of prostate cancer. *Medical Decision Making*. 19: 242-251.

Everts PAM, Mahoney CB, Hoffmann JJML, Schönberger JPAM, Box HAM, Zundert A and Knape JTA (2006). Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors*. 24(3):165–171.

Fernandez-Barbero JE, Galindo-Moreno P, Avila-Ortiz G, Caba O, Sanchez-Fernandez E and Wang HL (2006). Flow cytometric and morphological characterization of platelet-rich plasma gel. *Clin Oral Implants Res.*17: 687-693.

Fini M and Giardino R (2003). *In vitro* and *in vivo* tests for the biological evaluation of candidate orthopedic materials: benefits and limits. Journal of *Applied Biomaterials & Biomechanics*. 1:155-163.

Fiske J, Davis DM, Frances C and Gelbier S (1998). The emotional effects of tooth loss in edentulous people. *Br Dent J.*184(2):90-93

Fiske J, Davis DM, Leung KCM, McMillan AS and Scott BJJ (2001). The emotional effects of tooth loss in partially dentate people attending prosthodontic clinics in dental schools in England, Scotland and Hong Kong: A preliminary investigation. *International Dental Journal*. 51(6):457-462.

Florian M, Christian S, Joselyne SF, Torsten M, Olaf B, Ulrich J, Thomas K and Reiner B (2005). The impact of tooth loss on general health related quality of life among elderly Promeranians: Results from the study of health in Promerania. *Int J Prosthodont*. 18:414-419.

Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB and Rodeo SA (2009). Platelet-rich plasma: from basic science to clinical applications. *Am J Sports Med*. 37 (11): 2259-2272.

Freymiller EG and Aghaloo TL (2004). Platelet-rich plasma: ready or not? *J Oral Maxillofac Surg*. 62:484-488.

Gailain MWA and Latif TA (2015). The effect of platelet rich plasma on osseointegration period of dental implants. *J Bagh College Dentistry*. 27(4):101-106

Galois L and Mainard D (2004). Bone ingrowth into two porous ceramics with different pore sizes: an experimental study. *Acta Orthop Belg*. 70:598-603.

Gandhi A, Bibbo C, Pinzur M and Lin SS (2005). The role of platelet-rich plasma in foot and ankle surgery. *Foot Ankle Clin*. 10(4): 621-37.

Gentile P, Bottini DJ, Spallone D, Curcio BC and Cervelli V (2010). Application of platelet-rich plasma in maxillofacial surgery: clinical evaluation. *J Craniofac Surg*. 21(3):900-904

Georgakopoulos I, Tsantis S, Georgakopoulos P, Korfiatis P, Fanti E, Martelli M, Costaridou L, Petsas T, Panayiotakis G, and Martelli FS (2014). The impact of Platelet Rich Plasma (PRP) in osseointegration of oral implants in dental panoramic radiography: texture based evaluation. *Clin Cases Miner Bone Metab.* 11(1): 59-66.

Ghahroudi AAR, Homayouni A, Rokn AR, Kia F, Kharazifard MJ and Khorsand A (2015). Frequency of Dental Implants Placed in the Esthetic Zone in Dental Clinic of Tehran University: A Descriptive Study. *J Dent (Tehran)*. 12(12): 906–912.

Giori NJ, Ryd L and Carter DR (1995). Mechanical influences on tissue differentiation at bone-cement interfaces. *J Arthroplasty.* 10:514-522.

Glauser R, Lundgren AK, Gottlow J, Sennerby L, Portmann N, Ruhstaller P and Hämmerle CH (2003). Immediate occlusal loading of Brånemark TiUnite implants placed predominantly in soft bone: 1 year results of a prospective clinical study. *Clin Implan Dent Relat Res*. 5(1): 47-56.

Gonshor A (2002). Technique for producing platelet-rich plasma and platelet concentrate: background and process. *Int J Periodontics Restorative Dent.* 22(6): 547-557.

Goodacre CJ, Benal G, Kitichai RK and Kan JY (2003). Clinical complications in fixed prosthodontics. *J Prosthet Dent.* 90 (1): 31-41.

Gotfredsen K and Walls AW (2007). What dentition assures oral function? *Clin Oral Implants Res*. 18 (Suppl 3):34-40

Green D (1998). Plastic and Reconstructive Surgery. *Journal of American Society of Plastic Surgeons.* 101(4).

Griffin XL, Smith CM and Costa ML (2009). The clinical use of platelet-rich plasma in the promotion of bone healing: a systematic review. *Injury*. 40(2): 158-162.

Gulden M (1997). Manufacturer of the Periotest. Available at www.med-gulden.com, Accessed on 17/04/2017.

Hashem AA, Claffey NM and Connell BO (2006). Pain and Anxiety Following the Placement of Dental Implants. *The International Journal of Oral & Maxillofacial Implants*. 21(6): 943-950.

Henderson JL, Cupp CL, Ross EV, Shick PC, Keefe MA, Wester DC, Hannon T and McConnell D (2003). The effects of autologous platelet gel on wound healing. *Ear Nose Throat J.* 82(8): 598-602.

Hofmann AA, Bloebaum RD and Bachus KN (1997). Progression of human bone ingrowth into porous-coated implants. Rate of bone ingrowth in humans. *Acta Orthop Scand*. 68:161-166.

Hom-Lay W and Gustavo A (2007). Platelet Rich Plasma: Myth or Reality? *Eur J Dent*. 1(4): 192-194.

Hoque MM (2016). Sample size calculation: abc of research methodology and biostatistics, 2nd ed (revised): 155.

Irish JD (2004). A 5,500-year-old artificial human tooth from Egypt: A Historical Note. *Int J Oral Maxillofacial Implants*.19(5)

Issa JPM, Tiossi R, Mello ASdaS, Lopes RA, DiMatteo MAS and Iyomasa MM (2007). PRP: A possibility in Regenrative Therapy. *International Journal of Morphology*. 25(3):587-590

Jakse N, Tangl S, Gilli R, Berghold A, Lorenzoni M, Eskici A, Haas R and Pertl C (2003). Influence of PRP on autogenous sinus grafts. An experimental study on sheep. *Clin Oral Implants Res.*14:578-583.

James LR, David AJ, Nicholas MR and James WF (2010). Platelet rich plasma to facilitate wound healing following tooth extraction. *Journal of Oral Implantology.*36 (1): 11-23.

Janes H and Pepe M (2006). The optimal ratio of cases to controls for estimating the classification accuracy of a biomarker. *Biostatistics*. 7 (3): 456-468.

Jivraj S and Chee W (2006). Treatment planning of implants in aesthetic zone. *Br. Dent J.* 201(1): 13-25.

Johansson CB and Albrektsson T (1991). A removal torque and histomorphometric study of commercially pure niobium and titanium implants in rabbit bone. *Clin Oral Implants Res*. 2:24-29.

John B and Lewis Steven L (1989). The Branemark implant system: clinical and laboratory procedures. *St. Louis: Ishiyaku Euro America.*

John B and Steven L (1989). The Branemark implant system: clinical and laboratory procedures. *St. Louis: Ishiyaku EuroAmerica*. ISBN 0-912791-62-4.

Kanth KL, Swamy DN, Mohan TK, Swarna C, Sanivarapu S and Pasupuleti M (2014). Determination of implant stability by resonance frequency analysis device during early healing period. *J NTR Univ Health Sci.* 3:169-75.

Kassolis JD and Reynolds MA (2005). Evaluation of the adjunctive benefits of platelet-rich plasma in subantral sinus augmentation. *J Craniofac Surg.* 16: 280-287.

Kathleen ML, PA-C and Alan D (2010). Platelet-rich plasma: support for its use in wound healing. *Yale J Biol Med.* 83(1):1-9.

Khairy NM, Shendy EE, Askar NA and El-Rouby DH (2012). Effect of platelet rich plasma on bone regeneration in maxillary sinus augmentation (randomized clinical trial) *Int J Oral Maxillofac Surg*. 42(2):249-255

Kim YH and Kim VE (1993). Uncemented porous-coated anatomic total hip replacement. Results at six years in a consecutive series. *J Bone Joint Surg Br*. 75:6-13.

Knighton DR, Doucette M, Fiegel VD, Ciresi K, Butler EL and Austin L (1998). The use of platelet derived wound healing formula in human clinical trials. *Prog Clin Biol Res.* 266:319-329.

Kujala S, Ryhanen J, Danilov A and Tuukkanen J (2003). Effect of porosity on the osteointegration and bone ingrowth of a weight-bearing nickel-titanium bone graft substitute. *Biomaterials.* 24:4691-4697.

Kurzweg H, Heimann RB, Troczynski T and Wayman ML (1998). Development of plasma-sprayed bioceramic coatings with bond coats based on titania and zirconia. *Biomaterials*. 19:1507-1511.

Lang NP and Berglundh T (2011). Working Group 4 of the Seventh European Workshop on Periodontology. Periimplant diseases: where are we now?-Consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol.* 38 (11):178-81.

Larsson C, Thomsen P, Aronsson BO, Rodahl M, Lausmaa J, Kasemo B and Ericson LE (1996). Bone response to surface-modified titanium implants: studies on the early tissue response to machined and electropolished implants with different oxide thicknesses. *Biomaterials*. 17:605-616.

Lavenus S, Louarn G, and Layrolle P (2010). Nanotechnology and Dental Implants. *Int J Biomater.* 2010:1-9.

Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM and Ince C (2007). Influence of the application of platelet-enriched plasma in oral mucosal wound healing. *Clin Oral Implants Res.*18(1):133-139.

Linder L, Obrant K and Boivin G (1989). Osseointegration of metallic implants. II. Transmission electron microscopy in the rabbit. *Acta Orthop Scand.* 60:135-139.

Lindh T, Gunne J, Tillberg A and Molin M (1998). A meta analysis of implants in partial edentulism. *Clin Oral Implants Res*. 9(2): 80-90.

Lindhe J and Meyle J (2008). Group D of European Workshop on Periodontology. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol.* 35(8):282-5.

Locker D and Slade G (1994). Association between clinical and subjective indicators of oral health status in an older adult population. *Gerodontology*. 11: 108-114.

Löe H (1967). The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol*. 38: 610-616.

Lucarelli E, Donati D, Cenacchi A and Fornasari PM (2004). Bone reconstruction of large defects using bone marrow derived autologous stem cells. *Transfus Apher Sci.* 30:169-174.

Manimaran and Saisadan (2010). Platelet rich rlasma in implant dentistry-current trends. *Journal of Indian Academy of Dental Specialist Research (JIADS)*. 1(3): 22-24.

Marco F, Milena F, Gianluca G and Vittoria O (2005). Periimplant osteogenesis in health and osteoporosis. *Micron*. 36:630-644.

Marei H, Mostafa MSE, Mohamed SH and Amr AE (2009). Enhancement of Osseointegration of Oral Implants utilizing Platelet rich Plasma Factors. *Clinical Oral Implant Res*. 55(22):1371

Marx RE (1999). Platelet Rich Plasma: A Source of Multiple Autologous Growth Factors for Bone Grafts. *In Tissue Engineering: Application in Maxillofacial Surgery and Periodontics* (eds: Lynch SE, Genco RJ, Marx RE). Chicago: Quintessence: 71-82.

Marx RE (2001). Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent.* 10(4):225-228.

Marx RE (2004). Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg.* 62(4):489-496.

Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE and Georgeff KR (1998). Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 85:638-646.

Masuda T, Yliheikkila P and Felton DC (1998). Generalizations regarding the process and phenomenon of osseointegration. Part I. In Vivo Studies. *The International Journal of Oral and Maxillofacial Implants.* 13:17-29.

Matusovits D (2009). *Investigation of the osseointegration of dental implants and different biomaterials used in guided tissue regeneration, PhD thesis*, University of Szeged

Mavrogenis AF, Dimitriou R, Parvizi J and Babis GC (2009) Biology of implant osseointegration. *J Musculoskelet Neuronal Interact.* 9(2):61-71.

Mazzucco L, Balbo V, Cattana E and Borzini P (2008). Platelet-rich plasma and platelet gel preparation using Plateltex. *Vox Sang.* 94(3):202-208.

McAleer JP, Sharma S, Kaplan EM and Persich G (2006). Use of autologous platelet concentrate in a nonhealing lower extremity wound. *Adv Skin Wound Care.* 19(7): 354-363.

McClarenc (2003). *Close to the Edge-Brånemark and the Development of Osseointegration*. Edited by: Elaine McClarence. Quintessence.

Mehta S and Watson JT (2008). Platelet rich concentrate: basic science and current clinical applications. *J Orthop Trauma*. 22(6):432–438.

Meyer U, Joos U, Mythili J, Stamm T, Hohoff A, Fillies T, Stratmann U and, Wiesmann HP (2004). Ultrastructural characterization of the implant/bone interface of immediately loaded dental implants. *Biomaterials.* 25:1959-1967.

Millington JT and Norris TW (2000). Effective treatment strategies for diabetic foot wounds. *J Fam Pract.* 49(11 Suppl.): S40-S48.

Misch CE (2011). Generic Root Form Component Terminology, *Contemporary Implant Dentistry, 3rd ed. eBook:* 26-38.

Mishra A and Pavelko T (2006). Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. The American Journal of Sports Medicine; 34 (11): 1774-1778.

Mishra A, Velotta J, Brinton TJ, Wang X, Chang S, Palmer O, Sheikh A, Chung J, Young PC, Robbins R and Fschbein M (2010). RevaTen platelet-rich plasma improves cardiac function after myocardial injury. Cardiovasc Revasc Med. 12 (3): 158-63.

Mishra A, Woodall J and Vieira A (2009). Treatment of tendon and muscle using platelet-rich plasma. *Clinics in Sports Medicine*. 28(1):113-25.

Mohammadi S, Esposito M, Cucu M, Ericson LE and Thomsen P (2001). Tissue response to hafnium. *J Mater Sci Mater Med*. 12:603-611.

Mombelli A, Van Oosten MAC, Schürch E and Lang NP (1987). The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol*. 2:145-151.

Nandal S, Ghalaut P, Shekhawat H and Nagar P (2014). Osseointegration in Dental Implants: A Literature Review. *Indian Journal of Applied Research*. 4(7): 411-413.

Navins M, Giannobile WV, McGuire MK, Kao RT, Mellonig JT, Hinrichs JE, McAllister BS, Murphy KS, McClain PK, Nevins ML, Paquette DW, Han TJ, Reddy MS, Lavin PT, Genco RJ and Lynch SE (2005). Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *J Periodontol*. 76(12): 2205-2215

Nazaroglou I, Stavrianos C, Kafas P, Matoulas E, Upile T, Barlas I. and Jerjes W (2009). Radiographic evaluation of bone regeneration after the application of plasma rich in growth factors in a lower third molar socket: a case report. *Int. J Oral Maxillofac Implants*. 2: 9134

Neto RS, Pontes AEF, Wenzel A and Sakakura CE (2014). Patient Discomfort Following Single-Tooth Implant Placement: A Randomized Controlled Trial of Immediate vs. Conventional Tooth Restoration. *Journal of Oral Health and Dental Management (JOHDM)*. 13(2): 441-445.

Nikolidakis D and Jansen JA (2008). The biology of platelet-rich plasma and its application in oral surgery: literature review. *Tissue Eng Part B Rev.* 14(3):249-258.

Nowjack-Raymer R E and Sheiham A. (2003). Association of edentulism and diet and nutrition in U.S. adults. *J Dent Res*. 123-126.

Nygren H, Eriksson C and Lausmaa J (1997). Adhesion and activation of platelets and polymorphonuclear granulocyte cells at TiO_2 surfaces. *Lab Clin Med.* 129:35-46.

OSSTEM Surgical Manual (2006). Available at: http://www.osstem.com. Accessed on 15. 08. 2012

Padmanabhan TV and Gupta RK (2010). Comparison of Crestal Bone Loss and Implant Stability Among the Implants Placed With Conventional Procedure and Using Osteotome Technique: A Clinical Study. *Journal of Oral Implantology*. 36(6): 475-483.

Park JY and Davies JE (2000). Red blood cell and platelet interactions with titanium implant surfaces. *Clin Oral Implants Res*. 11:530-539.

Patil SH, Veena HR, Chkrasali M, Shah AC (2012). Dental Implant Maintenance-A Review. *International Journal of Dental Clinics*. 4(1):35-40

Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M and Yasui Y (2001). Phases of biomarker development for early detection of cancer. *Journal of the National Cancer Institute*. 93:1054-1061.

Petrungaro PS (2001). Using platelet-rich plasma to accelerate soft tissue maturation in esthetic periodontal surgery. *Compend Contin Educ Dent*. 22:729-732. 734-736.

Pietramaggiori G, Kaipainen A, Czeczuga JM, Wagner CT and Orgill DP (2006). Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds. *Wound Repair Regen*.14(5):573-580.

Pietrzak WS and Eppley BL (2005). Platelet rich plasma: biology and new technology. *J Craniofac Surg*. 16(6):1043–1054.

Pjetursson BE, Tan K, Lang NP, Bra¨gger U, Egger M and Zwahlen M (2004). A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. IV. Cantilever or extension FPDs. *Clin. Oral Impl. Res*.15: 667–676

Poeschl PW, Ziya-Ghazvini F, Schicho K, Buchta C, Moser D, Seemann R, Ewers R and Schopper C (2012). Application of platelet-rich plasma for enhanced bone regeneration in grafted sinus. *J Oral Maxillofac Surg*. 70:657-664.

Por YC, Shi L, Samuel M, Song C and Yeow VK (2009). Use of tissue sealants in face-lifts: a meta analysis. Aesthetic Plastic Surgery. 33(3): 336-339.

Puleo DA and Nancy A (1999). Understanding and controlling the bone-implant interface. *Biomaterials*. 20:2311-2321.

Rae T (1975). A study on the effects of particulate metals of orthopaedic interest on murine macrophages *in vitro*. *J Bone Joint Surg Br.* 57:444-450.

Rae T (1981). The toxicity of metals used in orthopaedic prostheses. An experimental study using cultured human synovial fibroblasts. *J Bone Joint Surg Br*. 63: 435-440.

Rajpal J, Gupta KK, Tandon P, Srivastava A, Chandra C (2014). Assessment of hard and soft tissue changes around Implants: A clinico-radiographic *in vivo* study. *J Dent Implant.* 4(2): 126-134.

Ring ME (1995a). A thousand years of dental implant: A definitive history-Part One. *Compendium of Continuing Educaation in Dentistry*. 16(10):1060-1069.

Ring ME (1995b). A thousand years of dental implant: A definitive history-Part Two. *Compendium of Continuing Educaation in Dentistry*. 16(11):1132-1142.

Rosenstiel SF, Land COMF, Fujimoto AIJ (2006). *Contemporary Fixed Prosthodontics*. Mosby Elsevier,Tokyo, Japan.

Rozman P and Bolta Z (2007). Use of platelet growth factors in treating wounds and soft-tissue injuries. *Acta Dermatovenerol Alp Panonica Adriat.* 16(4):156-165.

Saini GS and Goyal M (2012). Objective assessment of implants stability placed in fresh extraction socket using periotest device. *Int J Oral Implantol Clin Res*. 3(2):67-70.

Sammartino G, Tia M, Marenzi G, di Lauro AE, D'Agostino E and Claudio PP (2005). Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. *J Oral Maxillofac Surg.* 63: 766-770.

Samule EL (1994). *The role of growth factors in periodontal repair and regeneration*. Periodontal regeneration: current status and directions: 179-198.

Sanchez AR, Sheridan PJ and Kupp LI (2003). Is platelet-rich plasma the perfect enhancement factor? A current review. *Int J Oral Maxillofac Implants*.18: 93-103.

Schutle W (1988). A new field of application method: The occlusal-periodontal load can now be measured quantitatively. *Zahnaarztl Mitt*. 78(5): 1-11.

Sennerby L and Meredith N (2008). Implant stability measurements using resonance frequency analysis: biological and biomechanical aspects and clinical implications. *Periodontology2000.* 47(1): 51-66.

Sennerby L, Thomsen P and Ericson LE (1992). A morphometric and biomechanic comparison of titanium implants inserted in rabbit cortical and cancellous bone. *Int J Oral Maxillofac Implants*. 7: 62-71.

Sheiham A, Steele JG, Marcenes W and Al E (2001). Prevalence of impacts of dental and oral disorders and their effects on eating among older people: A national survey in Great Britain. *Community Dent Oral Epidemiol*. 29:195-203.

Silness J and Löe H (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand.* 22:121-135.

Soballe K (1993). Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs. *Acta Orthop Scand Suppl.* 255:1-58.

Soballe K, Overgaard S, Hansen ES, Brokstedt- Rasmussen H, Lind M and Bunger C (1999). A review of ceramic coatings for implant fixation. *J Long Term Eff Med Implants*. 9:131-151.

Spadaro JA, Albanese SA and Chase SE (1990). Electromagnetic effects on bone formation at implants in the medullary canal in rabbits. *J Orthop Res*. 8:685-693.

Sundgren JE, Bodo P, Lundstrom I, Berggren A and Hellem S (1985). Auger electron spectroscopic studies of stainless-steel implants. *J Biomed Mater Res*. 19: 663-671.

Tan K, Pjetursson BE, Lang NP and Chan ESY (2004). A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. III. Conventional FPDs. *Clin. Oral Impl. Res.*15; 654–666

Tang YQ, Yeaman MR and Selsted ME (2002). Antimicrobial peptides from human platelets. *Infect Immun.* 70(12): 6524-6533.

Thomsen P, Larsson C, Ericson LE, Sennerby L, Lausmaa J and Kasemo B (1997). Structure of the interface between rabbit cortical bone and implants of gold, zirconiumand titanium. *J Mater Sci Mater Med.* 8: 653-665.

Ucer C (2012). A Dentist's Guide to Implantology, *Association of Dental Implantology*

Walton JN, Gardner FM and Agar JR (1986). A survey of crown and fixed partial denture failures: length of service and reasons for replacement. *J Prosthet Dent.* 56(4):416-21

Weibrich G, Kleis WK, Kunz-Kostomanolakis M, Loos AH and Wagner W (2001). Correlation of platelet concentration in platelet-rich plasma to the extraction method, age, sex, and platelet count of the donor. *Int J Oral Maxillofac Implants.*16(5): 693-699.

Westphal RG (1984). Health risks to cytapheresis donors. *Clin Haematol.* 13(1): 289-301.

Whitman DH, Berry RL and Green DM (1997). Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg.* 55: 1294-1299.

Wikipedia (2009). The first patient, Scientific Dental Clinic. Available at: https://en.wikipedia.org/wiki/G%C3%B6sta_Larsson, accessed on 25-06-2009.

Wikipedia (2010a). Nobel Biocare: History. Available at: https://en. wikipedia. org/wiki/ G%C3%B6sta_Larsson, accessed on 13-06-2010.

Wikipedia (2010b). The History of Dental Implantation, implantru.com. Available at: https://en.wikipedia.org/wiki/G%C3%B6sta_Larsson, accessed on 13. 06. 2010.

Wikipedia (2011). Platelet rich plasma. http://en.wikipedia.org/wiki/ Platelet-rich_plasma, Accessed on January 5, 2012.

Wittenberg RH, Shea M, Swartz DE, Lee KS, White AA and Hayes WC (1991). Importance of bone mineral density in instrumented spine fusions. *Spine*. 16:647-652.

Wojtowicz A, Chaberek S, Urbanowska E and Ostrowski K (2007). Comparison of efficiency of platelet rich plasma, hematopoieic stem cells and bone marrow in augmentation of mandibular bone defects. *NY State Dent J*. 73:41-45.

Wrotniak M, Bielecki T and Gazdzik TS (2007). Current opinion about using the platelet-rich gel in orthopaedics and trauma surgery. *Ortop Traumatol Rehabil.* 9(3): 227-238.

Yu W, Wang J and Yin J (2011). Platelet-Rich Plasma: A Promising Product for Treatment of Peripheral Nerve Regeneration After Nerve Injury. *Int J Neurosci*. 121(4): 176-180.

Zarb GA (1983). Introduction to osseointegration in clinical dentistry. *J Prosthetic Dent*. 49: 824.

Zarb GA and Albrektsson T (1991). Osseointegration: a requiem for the periodontal ligament? *Int J Periodont Rest Dent*. 11: 88-91.

Zarb GA, Bolender LC, Eckert ES, Jacob FR, Fenton HA and Mericske SR (2005). *Prosthodontic Treatment for Edentulous Patients*, 12th edition, Mosby Elsevier, New Delhi.

APPENDICES

APPENDICES

Appendix 1

History Sheet

1. Particulars of the Patient

1.1. Name of the patient: .....

1.2. Age of the patient: .....

1.3. Sex of the patient: M/F

1.4. Religion: .....

1.5. Occupation: .....

1.6. Permanent Address: .....

1.6. Present Address: .....

1.7. Contact No.: .....

2.	Medical History (*circle YES or NO, which one applies)*	
2.1.	Are you now under the care of physician? If yes, what is the condition treated? ..... Name and address of Physician:	Yes / No
2.2.	Are you allergic or have you had a reaction to?	
	2.2.1. Alcohol	Yes / No
	2.2.2. Aspirin	Yes / No
	2.2.3. Latex	Yes / No
	2.2.4. Narcotics	Yes / No
	2.2.5.Barbiturates, sedatives Penicillin or other or sleeping pills	Yes / No
	2.2.6. Codeine or other	Yes / No
	2.2.7. Sulfa drugs	Yes / No
	2.2.8. Iodine	Yes / No
	2.2.9. Local anesthetics	Yes / No
	2.2.10. 0ther.....	Yes / No
2.3.	Have you had any serious illness, operation or been hospitalized in the last 5 years? If so, what was the illness/problem?	Yes / No

2.4.	Have you had any joint replacement surgery?	Yes / No

2.5.	Does your physician require you to premedicate with antibiotics for dental treatment? If so what medication.....	Yes / No
2.6.	Are you taking ANY medicine(s) including non-prescription? What medications are you taking (including blood thinners, osteoporosis medications	Yes / No
2.7.	Do you have cardiovascular disease - heart trouble, heart attack, angina, coronary insufficiency, coronary occlusion, high blood pressure, arteriosclerosis, stroke?	Yes / No
2.8.	Do you have damaged heart valves or artificial heart valves, including heart murmur, rheumatic heart disease?	Yes / No
2.9.	Have you ever had any treatment for a tumor or growth?	Yes / No
2.10.	Do you have any blood disorder such as anemia?	Yes / No
2.11.	Have you had abnormal bleeding?	Yes / No
2.12.	Have you ever required a blood transfusion?	Yes / No
2.13.	Do you have chest pain upon exertion?	Yes / No
2.14.	Are you ever short of breath after mild exercise or when lying down?	Yes / No
2.15.	Do your ankles swell?	Yes / No
2.16.	Do you have inborn heart defects?	Yes / No
2.17.	Do you have a cardiac pacemaker? Yes No	Yes / No
2.18.	Do you have or have you had any of the following diseases or problems?	
	2.18.1. AIDS or HIV	Yes / No
	2.18.2. Allergy	Yes / No
	2.18.3. Arthritis or painful swollen joint	Yes / No
	2.18.4. Asthma or Hey fever	Yes / No
	2.18.5. Cancer	Yes / No
	2.18.6. Diabetes	Yes / No
	2.18.7. Epilepsy or other neurological disease	Yes / No

	2.18.8.Fainting spells or seizer	Yes / No
	2.18.9. Hepatitis, Jaundice or Liver diseases	Yes / No
	2.18.10. Herpes (fever sores)	Yes / No
	2.18.11. Kidney trouble	Yes / No
	2.18.12. Low blood sugar	Yes / No
	2.18.13. Persistent cough or cough that produces blood	Yes / No
	2.18.14. Persistent diarrhea or recent weight loss	Yes / No
	2.18.15. Persistent swollen glands in neck	Yes / No
	2.18.16. Problems with mental health	Yes / No
	2.18.17. Problems with immune system	Yes / No
	2.18.19. Respiratory problems, emphysema, bronchitis etc.	Yes / No
	2.18.20. Sexually transmitted disease	Yes / No
	2.18.21. Sinus trouble	Yes / No
	2.18.22. Stomach ulcer	Yes / No
	2.18.23. Hyperthyroid	Yes / No
	2.18.24. Hypothyroid	Yes / No
	2.18.25. Tuberculosis	Yes / No
2.19.	Do you drink alcoholic beverages? If so, how much?	Yes / No
2.20.	Do you smoke or use tobacco products? If so how much?	Yes / No
2.21.	Do you have any disease, condition, or problem not listed above that you think I should know about? If so, explain	Yes / No
2.22.	Are you wearing contact lenses?	Yes / No
2.23.	Female Patients	
	2.23.1. Are you pregnant?	Yes / No
	2.23.2. Are you nursing?	Yes / No
	2.23.3. Are you taking birth control pills?	Yes / No
3.	Dental History	Yes / No
3.1.	Chief dental complaint (s).	
3.2.	Causes of tooth loss	
3.3.	History of tooth loss	
3.3.	Duration of edentulism	

3.4.	Have you had any trouble associated with any previous dental treatment? Explain.....	Yes / No
3.5.	Do you suffer from any TMJ problems? Explain	Yes / No
3.6.	Are you wearing removable dental appliances?	Yes / No
3.7.	Are you wearing removable dental appliances?	Yes / No
3.8.	Do you any idea about dental implant?	Yes / No

I certify that 1 have read and understand the above. I acknowledge that my questions, if any, about the inquiries set forth above have been answered to my satisfaction. I will not hold my dentist, or any other member of his/her staff, responsible for any errors or omissions that I may have made in the completion of this form.

_____

Signature of patient/guardian (Relationship to patient)

_____

Signature of Researcher

Date _____

Appendix 2

Informed Consent for Dental Implant Placement

Patient's name.....Date of birth.....

You have the right and the obligation to make decisions regarding your healthcare. Your dentist can provide you with the necessary information and advice, but as a member of the healthcare team, you must participate in the decision-making process. This form will acknowledge your consent to treatment recommended by your dentist.

1. I request and authorize Dr. or his/her associates or assistants to perform the surgical placement of dental implants upon me. This procedure has been recommended to me by my dentist as an option to replace my natural teeth.

Dental implants are metal anchors put inside the jawbone underneath the gum line. Small posts are attached to the implants and artificial teeth or dentures are fastened to the posts. Most patients need two surgical procedures to install the implants. The first procedure involves drilling small holes into the jawbone and placing the anchors. A temporary denture may be worn for a few months while the anchors bond with the jawbone and the gums and bone heal. The second procedure will uncover the implants to allow for attachment of the posts. After the posts are in place, the replacement teeth, in the form of fixed or removable bridgework or a denture, are fastened to the posts. Depending on the condition of the mouth, bone grafting or guided tissue regeneration also might be necessary to install the anchors and posts. The potential benefits of this procedure include the replacement of missing natural teeth or

supporting dentures. I authorize placement of implants in the areas of teeth _____.

2. I have chosen to undergo this procedure after considering the alternative forms of treatment for my condition, which include no treatment at all, complete or partial dentures, or fixed or removable bridges. Each of these alternative forms of treatment has its own potential benefits, risks and complications which have been explained to me.

3. I consent to the administration of anesthesia or other medications before, during or after the procedure by qualified personnel. I understand that all anesthetics or sedation medications include the very rare potential of risks or complications, such as damage to vital organs including the brain, heart, lungs, liver and kidneys; paralysis; cardiac arrest; and/or death from both known and unknown causes.

4. I understand that there are potential risks, complications and side effects associated with any dental procedure. Although it is impossible to list every potential risk, complication and side effect, I have been informed of some of the possible risks, complications and side effects of dental implant surgery. These could include but may not be limited to the following:

- Postoperative pain, discomfort and swelling
- Bleeding
- Postoperative infection
- Injury or damage to adjacent teeth or roots of the teeth

- Injury or damage to nerves in the lower jaw, causing temporary or permanent numbness and tingling or pain of the chin, lips, cheek, gums or tongue
- Restricted ability to open the mouth because of swelling and muscle soreness or stress on the joints in the jaw-temporomandibular joint (TMJ) syndrome
- Fracture of the jaw
- Bone loss of the jaw
- Penetration into the sinus cavity
- Mechanical failure of the anchors, posts, or attached teeth
- Failure to implant itself
- Allergic or adverse reaction to any medications

Most of these risks, complications or side effects are not serious and do not occur frequently. Although these risks, complications and side effects occur only very rarely, they do sometimes occur and cannot be predicted or prevented by the dentist performing the procedure. Although most procedures have good results, I acknowledge that no guarantee has been made to me about the results of this procedure or the occurrence of any risks, complications or side effects.

These potential risks and complications could result in the need to repeat the procedures; remove the implants; or undergo additional dental, medical or surgical treatment or procedures, hospitalization or blood transfusions. Very rarely, the potential risk and complications could result in permanent numbness, disability or death. I recognize that during the course of treatment, unforeseeable conditions may require additional

treatment or procedures. I request and authorize my dentist and other qualified medical personnel to perform such treatment as required.

5. I certify that I have read or had read to me the contents of this form. I have read or had read to me and will follow any patient instructions related to this procedure. I understand the potential risks, complications and side effects involved with any dental treatment or procedure and have decided to proceed with this procedure after considering the possibility of both known and unknown risks, complications, side effects and alternatives to the procedure. I declare that I have had the opportunity to ask questions and all of my questions have been answered to my satisfaction.

_____ Date _____

Patient /legally authorized representative signature

_____ Relationship _____

Printed name if signed on behalf of the patient

Appendix 3

Data Collection Sheet

ID No: .....Date: .....

Name: .....Age: .....Sex: M / F

Record of pain

Recording time	Patient of study group			Patient of control group		
	Present	Absent	VASS	Present	Absent	VASS
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						

Record of Swelling

Recording time	Patient of study group			Patient of control group		
	Present	Absent	VASS	Present	Absent	VASS
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Day 9						

Record of Imagistic Value

Recording time	Implant in study group				Implant in control group			
	Mesial	Distal	Apical	Grade	Mesial	Distal	Apical	Grade
Week 1								
Week 4								
Week 8								
Week 12								
Week 16								

Record of Vertical Bone Loss by Direct Measurement

Implant of Study group	Bone height= from top of implant to top of crestal bone (mm)			Implant of Study group	Bone height= from top of implant to top of crestal bone (mm)		
	1st Surgery	2nd Surgery	Bone loss		1st Surgery	2nd Surgery	Bone loss
	x	y	x–y		x	y	x–y

Record of Vertical Bone Loss by X-ray Tracing

Implant of Study group	Bone height= from top of implant to top of crestal bone (mm)			Implant of Study group	Bone height= from top of implant to top of crestal bone (mm)		
	1st Surgery	2nd Surgery	Bone loss		1st Surgery	2nd Surgery	Bone loss
	x	y	x–y		x	y	x–y

Record of Horizontal Bone Loss by Direct Measurement

Implant of Study group	Bone width = from buccal to lingual cortical plate (mm)			Implant of Study group	Bone width = from buccal to lingual cortical plate (mm)		
	1st Surgery	2nd Surgery	Bone loss		1st Surgery	2nd Surgery	Bone loss
	x	y	x–y		x	y	x–y

Record of Horizontal Bone Loss by Model Analysis

Implant of Study group	Bone width = from buccal to lingual cortical plate (mm)			Implant of Study group	Bone width = from buccal to lingual cortical plate (mm)		
	1st Surgery	2nd Surgery	Bone loss		1st Surgery	2nd Surgery	Bone loss
	x	y	x–y		x	y	x–y

Record of Stability by Periotest Value

Implant of Study group	Periotest Value			Implant of Study group	Periotest Value		
	1st Surgery	2nd Surgery	Value changed		1st Surgery	2nd Surgery	Value changed
	x	y	x–y		x	y	x–y

Record of Implant Stability Quotient (ISQ)

Implant of Study group	ISQ Value			Implant of Study group	ISQ Value		
	1st Surgery	2nd Surgery	Value changed		1st Surgery	2nd Surgery	Value changed
	x	y	x–y		x	y	x–y

Record of Periimplant Indices after 1year of loading

Periimplant Indices	Implant in study group			Implant in control group		
	Baseline	After 1 year	Difference	Baseline	After 1 year	Difference
PI (mm)	x	y	x–y	x	y	x–y
BOP	x	y	x–y	x	y	x–y
PPD (mm)	x	y	x–y	x	y	x–y

Appendix 4
Certificate of Ethical Clearance

UNIVERSITY OF RAJSHAHI
INSTITUTE OF BIOLOGICAL SCIENCES
Rajshahi 6205, Bangladesh
Tel: (880-721) 750928, Cell. +880-01556312361, Fax + 880-721-711117, +880-721-750064
Email: director_ibsc@ru.ac.bd, yeasmin_bio@yahoo.com, http: // www. ibsc-ru.org

Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes and Living Natural Sources

(Approved in the Resolution No. of the 71th meeting of the Board of Governors of the Institute of Biological Sciences and Resolution No. 57 of the 433th meeting of the Syndicate of the University of Rajshahi)

Memo No. 44 /320/IAMEBBC/IBSc 26December,2013

Certificate

This is to certify that the project title **"EFFECT OF PLATELET RICH PLASMA ON OSSEOINTEGRATION OF DENTAL IMPLANT"** Submitted by Md. Amzad Hossain, PhD Fellow, Institute of Biological Sciences, University of Rajshahi has been approved by the IAMEBBC in its resolution no. 5 of the 5th meeting held on 26December, 2013.

Name of Chairman: Prof. Dr. Tanzima Yeasmin

Signature with Date 26.12.13

Appendix 5

Mean age of the patients based on sex (n=288)

Sex	Frequency (%)	Age (years) [Mean ± SD]	df	t	P value
Male	152 (52.78)	47.21±14.17	286	0.78	0.453^{ns}
Female	136 (47.22)	45.99±13.36			
Total	288	46.64±13.78			

Unpaired 't' test was done to measure the level of significance.

^{ns} Not significant (P > 0.05)

Appendix 5a

Distribution of frequency and percentage of patients based on sex (n=288).

Sex	Study group (n_1=144) [n(%)]	Control group (n_2=144) [n(%)]	df	χ^2	P value
Male	76 (52.78)	76 (52.78)	1	0.000	1.000^{ns}
Female	68 (47.22)	68 (47.22)			
Total	144 (100.0)	144 (100.0)			

Chi-square test was done to measure the level of significance. Difference of frequency and percentage between male and female patients was not statistically significant.

^{ns} Not significant (P > 0.05).

Appendix 6

Distribution of patients based on age (n=288)

Age (years)	Male n (%)	Female n (%)	Total n (%)
22 - 32	24 (8.3)	22 (7.6)	46 (15.9)
33 - 42	36 (12.5)	34 (11.8)	70 (24.3)
43 - 52	40 (13.9)	38 (13.2)	78 (27.1)
53 - 62	26 (9.0)	22 (7.6)	48 (16.6)
63 - 72	16 (5.6)	14 (4.9)	30 (10.5)
73 - 82	10 (3.5)	6 (2.1)	16 (5.6)
Total	152 (52.8)	136 (47.2)	288 (100)

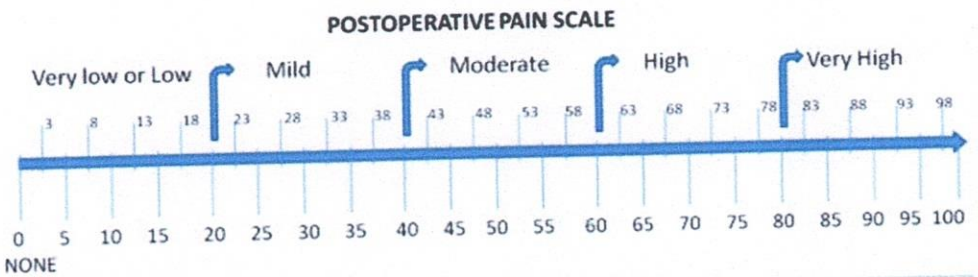
Appendix 7

Evaluation of postoperative pain of the patients (n=288)

Days	Study group (n_1=144)			Control group (n_2=144)		
	Present	Absent	VAS score	Present	Absent	VAS score
Day 1	144 (100.0)	0 (0.0)	22.67 ± 14.19	144 (100.0)	0 (0.0)	25.49 ± 13.42
Day 2	108 (75.0)	36 (25.0)	15.72 ± 13.92	116 (80.6)	28 (19.4)	18.46 ± 12.87
Day 3	72 (50.0)	72 (50.0)	6.00 ± 7.95	85 (59.0)	59 (41.0)	9.44 ± 10.85
Day 4	50 (34.7)	94 (65.3)	3.13 ± 5.41	60 (41.7)	84 (58.3)	5.42 ± 8.58
Day 5	30 (20.8)	114 (79.2)	1.25 ± 2.61	45 (31.3)	99 (68.8)	2.86 ± 4.53
Day 6	15 (10.4)	129 (89.6)	0.42 ± 1.31	35 (24.3)	109 (75.7)	1.75 ± 3.93
Day 7	0 (0.0)	144 (100.0)	0.00 ± 0.00	14 (9.7)	130 (90.3)	0.58 ± 2.09
Day 8	0 (0.0)	144 (100.0)	0.00 ± 0.00	0 (0.0)	144 (100.0)	0.00 ± 0.00

Appendix 7a

Visual Analogue Scale (VAS) for Postoperative Pain



Please, select a point on the scale indicating the level of pain you are feeling or felt in the indicated period. The number **0 indicates no pain** and **100 the worst pain** possible and felt in the period.

Appendix 8

Evaluation of postoperative swelling of the patients (n=288).

Reporting days	Study group (n_1=144)			Control group (n_2=144)		
	Present	Absent	VRS score	Present	Absent	VRS score
Day 1	105 (72.9)	39 (27.1)	2.31 ± 1.05	114 (79.2)	30 (20.8)	2.51 ± 1.04
Day 2	114 (79.2)	30 (20.8)	2.26 ± 0.90	134 (93.1)	10 (6.9)	2.85 ± 0.94
Day 3	108 (75.0)	36 (25.0)	2.28 ± 0.90	116 (80.6)	28 (19.4)	2.82 ± 1.11
Day 4	104 (72.2)	40 (27.8)	2.14 ± 0.82	110 (76.4)	34 (23.6)	2.64 ± 1.10
Day 5	64 (44.4)	80 (55.6)	1.51 ± 0.63	94 (65.3)	50 (34.7)	2.32 ± 1.13
Day 6	44 (30.6)	100 (69.4)	1.31 ± 0.46	69 (47.9)	75 (52.1)	1.99 ± 1.15
Day 7	20 (13.9)	124 (86.1)	1.14 ± 0.35	64 (44.4)	80 (55.6)	1.58 ± 0.72
Day 8	0 (0.0)	144 (100.0)	1.00 ± 0.00	24 (16.7)	120 (83.3)	1.17 ± 0.37
Day 9	0 (0.0)	144 (100.0)	1.00 ± 0.0	0 (0.0)	144 (100.0)	1.00 ± 0.0

Appendix 8a

Verbal Rating Scale (VRS) for postoperative swelling

Score	Interpretation
1	absence of swelling
2	intra-oral swelling in surgical zone
3	extra-oral swelling in surgical zone
4	extra-oral swelling extended beyond surgical zone

Appendix 9

Evaluation imagistic values of implants (n=244)

Groups	Range of value	Imagistic values				
		1st week n (%)	4th week n (%)	8th week n (%)	12th week n (%)	16th week n (%)
Study Group	-3	144 (100.0)	20 (13.9)	0 (0.0)	0 (0.0)	0 (0.0)
	-2	0 (0.0)	70 (48.6)	30 (20.8)	0 (0.0)	0 (0.0)
	-1	0 (0.0)	54 (37.5)	24 (16.7)	0 (0.0)	0 (0.0)
	1	0 (0.0)	0 (0.0)	40 (27.8)	0 (0.0)	0 (0.0)
	2	0 (0.0)	0 (0.0)	20 (13.9)	20 (13.9)	0 (0.0)
	3	0 (0.0)	0 (0.0)	30 (20.8)	24 (16.7)	15 (10.4)
	4	0 (0.0)	0 (0.0)	0 (0.0)	60 (41.7)	24 (16.7)
	5	0 (0.0)	0 (0.0)	0 (0.0)	40 (27.8)	60 (41.7)
	6	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	45 (31.3)
Control Group	-3	144 (100.0)	72 (50.0)	40 (27.8)	0 (0.0)	0 (0.0)
	-2	0 (0.0)	40 (27.8)	30 (20.8)	0 (0.0)	0 (0.0)
	-1	0 (0.0)	32 (22.2)	24 (16.7)	20 (13.9)	0 (0.0)
	1	0 (0.0)	0 (0.0)	40 (27.8)	20 (13.9)	10 (6.9)
	2	0 (0.0)	0 (0.0)	10 (6.9)	60 (41.7)	30 (20.8)
	3	0 (0.0)	0 (0.0)	0 (0.0)	44 (30.6)	104 (72.2)
	4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	6	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Quantification method was adopted by grading on scale from –3 to +3, where,

-3 = extended radiolucency, extended resorption; -2 = medium radiolucency, medium resorption; -1= minimum radiolucency, minimum resorption; 0 = no change; +1= minimum radio-opacity, minimum osteocondensation; +2 = medium radio-opacity, medium osteocondensation; +3 = extended radio-opacity, extended osteocondensation.

The grading was made in 3 regions viz., mesial, disatal and apical. Reference value was obtained by summing up the 3 grades. The reference range was between -3 and +6.

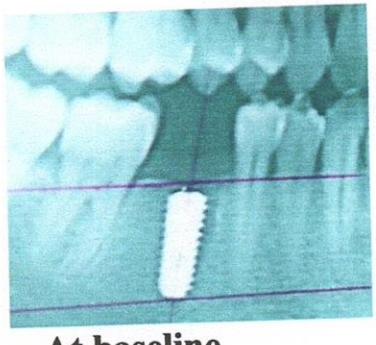
Appendix 10

Evaluation of vertical bone height of implants (n=244)

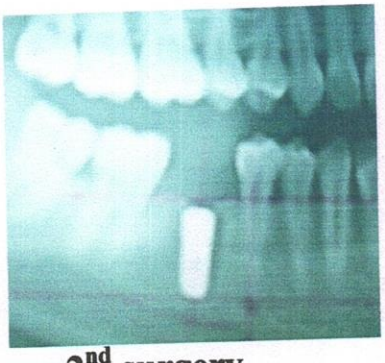
Groups	Range of value	Mean bone height (mm)	
		1st surgery (baseline) n (%)	2nd surgery n (%)
Study Group	0.5		30 (20.8)
	1.0	24 (16.7)	36 (25.0)
	1.5	26 (18.1)	34 (23.6)
	2.0	30 (20.8)	30 (20.8)
	2.5	32 (22.2)	14 (9.7)
	3.0	32 (22.2)	
Control Group	0.5		60 (41.7)
	1.0	26 (18.1)	64 (44.4)
	1.5	24 (16.7)	20 (13.9)
	2.0	28 (19.4)	0 (0.0)
	2.5	36 (25.0)	0 (0.0)
	3.0	30 (20.8)	

Appendix 10a

Method of vertical bone measurement by X-ray tracing



At baseline



2nd surgery

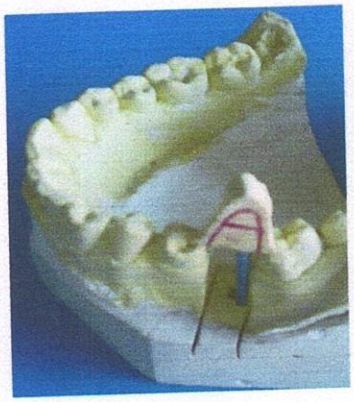
Appendix 11

Evaluation of horizontal bone around implants (n=288)

Groups	Range of value	Mean bucco/labio-lingual bone width (mm)	
		1st surgery (baseline) n (%)	2nd surgery n (%)
Study Group	5	20 (13.9)	40 (27.8)
	6	25 (17.4)	50 (34.7)
	7	55 (38.2)	40 (27.8)
	8	30 (20.8)	14 (9.7)
	9	14 (9.7)	0 (0.0)
Control Group	5	22 (15.3)	50 (34.7)
	6	20 (13.9)	75 (52.1)
	7	60 (41.7)	10 (6.9)
	8	30 (20.8)	9 (6.3)
	9	12 (8.3)	0 (0.0)

Appendix 11a

Cast analysis to measure horizontal bone



Bone width = total buccolingual width – soft tissue thickness

Appendix 12

Evaluation of implant stability by periotest values (n=288)

Groups	Range of values	Mean periotest value	
		Study group n (%)	Control group n (%)
1st surgery	9	40 (27.8)	50 (34.7)
	10	50 (34.7)	75 (52.1)
	11	40 (27.8)	10 (6.9)
	12	14 (9.7)	9 (6.3)
2nd surgery	-7	20 (13.9)	0 (0.0)
	-6	50 (34.7)	20 (13.9)
	-5	30 (20.8)	30 (20.8)
	-4	40 (27.8)	40 (27.8)
	-3	4 (2.8)	24 (16.7)
	-2	0 (0.0)	30 (20.8)

Appendix 12a

Periotest (PT) values used in the study

PT value range	Interpretation
–8 to 0	Good osseointegration, the implant is well osseointegrated and can be loaded
1 to 9	Clinical examination is required; in most cases, the implant loading is not yet possible
10 to 50	Osseointegration is insufficient, implant must not be loaded

Appendix 13

Evaluation of implant stability quotient (ISQ) (n=288)

Groups	Values	ISQ	
		Study group n (%)	Control group n (%)
1st surgery	30	44 (30.6)	0 (0.0)
	31	0 (0.0)	44 (30.6)
	33	0 (0.0)	50 (34.7)
	34	50 (34.7)	0 (0.0)
	36	50 (34.7)	50 (34.7)
2nd surgery	60	0 (0.0)	44 (30.6)
	61	0 (0.0)	50 (34.7)
	63	0 (0.0)	50 (34.7)
	80	44 (30.6)	0 (0.0)
	82	50 (34.7)	0 (0.0)
	83	50 (34.7)	0 (0.0)

Appendix 14

Evaluation of Periimplant Indices (n=180).

Plaque Index (PI)

Evaluation period	Value	Plaque Index	
		Study group n (%)	Control group n (%)
1st surgery	0	90 (100.0)	90 (100.0)
2nd surgery	0	40 (44.4)	20 (22.2)
	1	50 (55.6)	40 (44.4)
	2	0 (0.0)	30 (33.3)

Bleeding on Probing (BOP)

Evaluation period	Value	BOP	
		Study group n(%)	Control group n(%)
1st surgery	0	90 (100.0)	90 (100.0)
2nd surgery	0	60 (66.7)	30 (33.3)
	1	30 (33.3)	30 (33.3)
	2	0 (0.0)	30 (33.3)

Periimplant Probing Depth (PPD)

Evaluation period	value	PPD	
		Study group n(%)	Control group n(%)
1st surgery	1	40 (44.4)	50 (55.6)
	2	50 (55.6)	40 (44.4)
2nd surgery	1.5	40 (44.4)	20 (22.2)
	2.5	50 (55.6)	40 (44.4)
	3	0 (0.0)	30 (33.3)

Appendix 14a

The periimplant indices used in the present study

Plaque index (PI)

Score	Mombelli et al. 1987 (mPI)
0	No detection of plaque
1	Plaque can be recognized by running a probe across the smooth surface of implant
2	Plaque can be seen by naked eye
3	Abundance of soft matter

Bleeding on Probing (BOP)

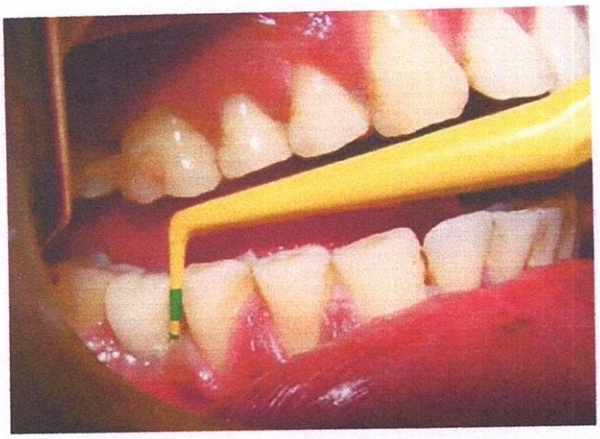
Score	Mombelli et al. 1987 (mGI)
0	No bleeding when a periodontal probe is passed along the mucosal margin adjacent to the implant
1	Isolated bleeding spots visible
2	Blood forms a confluent red line on mucosal margin
3	Heavy or profuse bleeding

Peri-implant Probing Depth (PPD)

The value depends on the gingival of abutment used for restoration of implant. Peri-implant probing depth was calculated using periodontal probe on mesial, distal, lingual and buccal sites and then the mean probing depth was calculated. In the present study, all abutments were selected with the gingival height ranged from 1 to 2 mm. So, the baseline periimplant probing depth was 1-2 mm.

Appendix 15

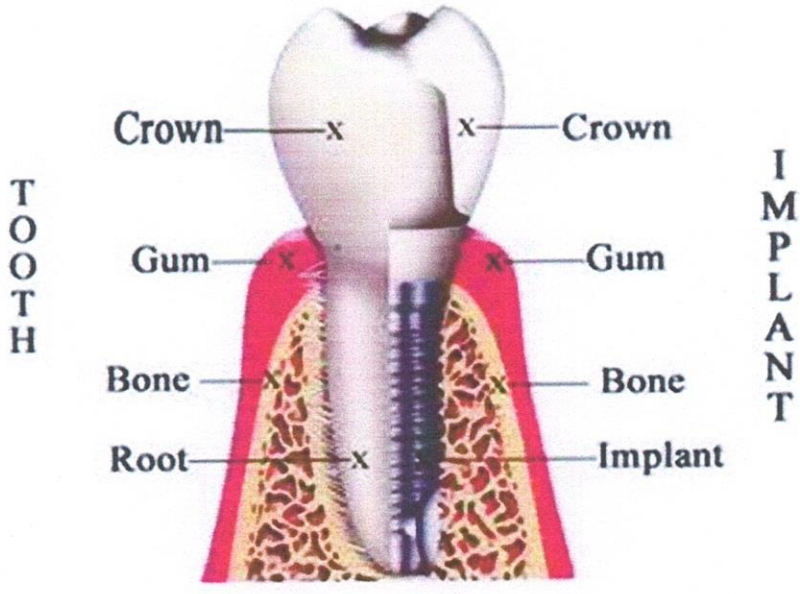
Measurement of Periimplant Probing Depth (PPD)



Plastic Graduated Perioprobe was used to measure PPD

Appendix 16

Comparative Anatomy of Tooth and Implant

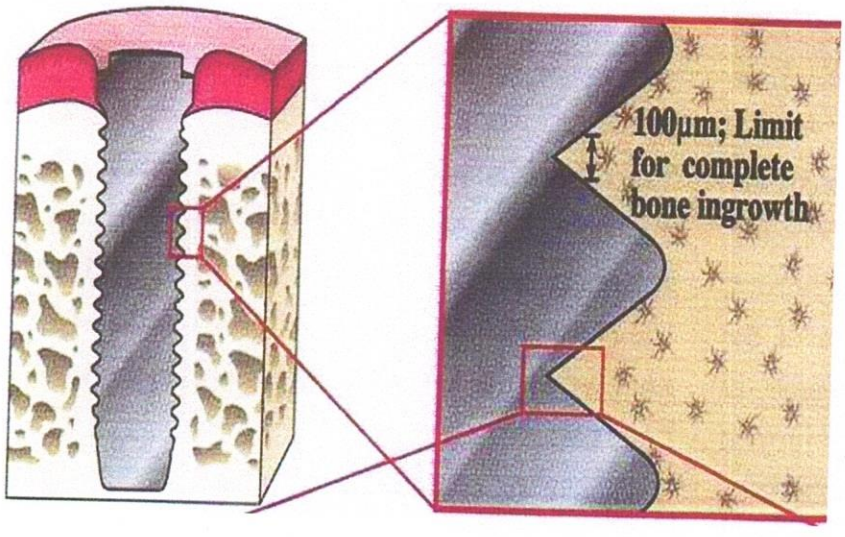


Appendix 17

An Implant Fixture

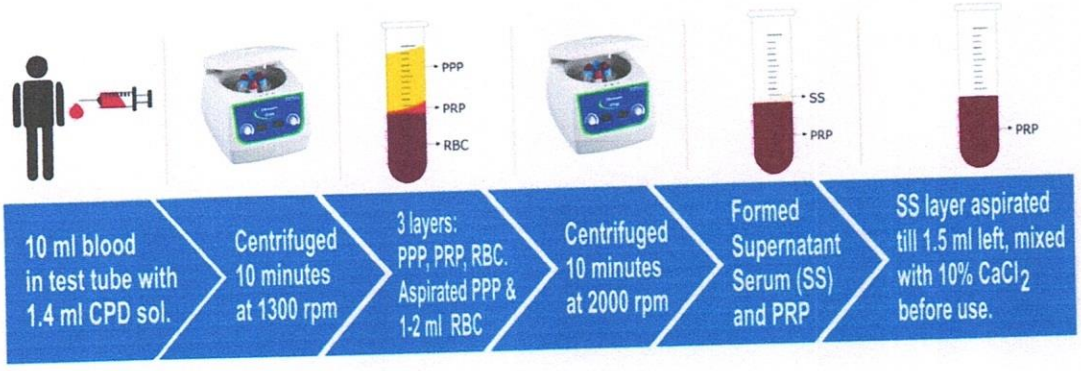
Appendix 18

Histological Feature of Osseointegration



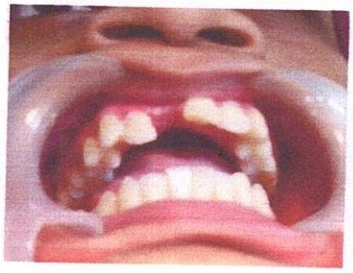
Appendix 19

Steps of platelet rich plasma (PRP) preparation

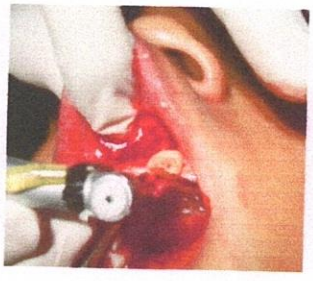


Appendix 20

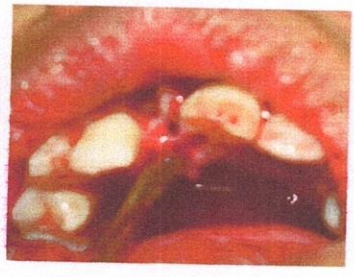
Steps for surgical placement of Implant



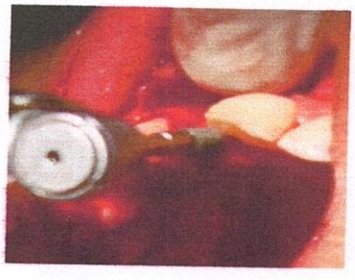
Edentulous space



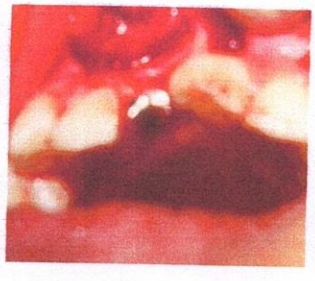
Osteotomy is being done



Oseotom Complete



Implant is being Inserted



Implant Placed

Suturing