Effect of age on expression of osteoclastogenic bone biomarkers in periodontal surgical treatment outcomes

Priyanka Gupta[1], Rameshwari Singhal[1], Pavitra Rastogi[1], Nand Lal[1], Anjani Kumar Pathak[1], Shivani Pandey[2], Abbas Ali Mahdi[2]

[1]King George's Medical University, Faculty of Dental Sciences, Department of Periodontology, Lucknow, UP, INDIA. [2]King George's Medical University, Faculty of Dental Sciences, Department of Biochemistry, Lucknow, UP, INDIA.

Abstract

Aim: Age is not considered a prognostic determinant in surgical periodontal treatment planning even though it is a non-modifiable risk factor. Present study explores the effect of age on bone coupling mechanism at molecular level through Osteoprotegrin (OPG) and Receptor activator of nuclear factor kappa-B ligand (RANKL) following surgical pocket therapy in periodontitis.

Methods: Forty-five generalized periodontitis patients were divided for surgical treatment into three groups based on age (in years): Group1 (Youth; 16-19); Group 2 (Adult; 25-45); Group 3 (Old; >60). Clinical parameters were evaluated at baseline, 12, and 24 weeks. OPG, RANKL, and its ratio were assessed using Enzyme-linked immunosorbent assay at baseline and 24 weeks.

Results: Stringent oral hygiene was maintained in Youth, Adult, and Old age-groups at all-time intervals. At 24 weeks, clinical and bone biomarker levels improved from baseline but the difference was not statistically significant across different age groups ($p>0.05$).

Conclusion: Age has no impact on surgical outcomes at the clinical or molecular bone level as long as oral-hygiene is well maintained and plaque accumulation is limited in post-surgical healing phase.

Keywords: Age groups; periodontitis; OPG; RANK Ligand; risk factors.

Introduction

Microbial plaque accounts for only 20% risk of individual developing periodontitis (Golub *et al*., 1997). Remaining 80% risk is associated with other modifiable, non-modifiable, and predisposing factors (Belibasakis, 2018; O'Connor *et al*., 2020). Evidence regarding effect of individual risk factors on disease progression and treatment is lacking. In today's era of personalized periodontal therapy, assessment of individual risk factors; their role in disease progression, and effect on treatment outcomes need to be understood for predictable, preventive, practical, and plausible periodontal therapy (Kornman and Duff, 2012).

Host response is a major factor in governing outcomes of periodontal disease progression or remission (Silva *et al*., 2015). Dysbiotic plaque leads to activation of host inflammatory-immune response, the release of pro-inflammatory cytokines (IL-1β &TNF-α), subsequently resulting in activation of T and B lymphocytes (Kayal, 2013). T-cells being an important regulator of bone metabolism, activate macrophages to initiate osteoclastogenesis (Brunetti *et al*., 2005). T-cells directly express receptor activator of nuclear factor κB ligand (RANKL) through pro-resorptive cytokines (IL-1, IL-6, IL-11). Levels of bone

Correspondence to: Rameshwari Singhal
E-mail: rameshwarisinghal@kgmcindia.edu

biomarkers reach a peak during pubertal growth spurt, followed by a rapid decline to adult levels (Weaver *et al*., 1997; Rauchenzauner *et al*., 2007).

Severity and extent of periodontal disease increase with age progression (Rabei *et al*., 2019; Al-Nasser *et al*., 2020). Aging has detrimental effect on stimulators of bone formation and repair. Immune parameters decline with increase in age (Peters *et al*., 2019). Age-related immune changes, known as immunosenescence, are associated with accumulation of subclinical pro-inflammatory factors. Inflammaging term was coined to indicate chronic, low-grade inflammation that develops with age and predicts susceptibility to age-related pathologies (Franceschi, 2000). Adolescent and young age are characterized by fluctuations in hormone levels and changes in gonadotrophic hormone levels with onset of puberty. These changes can modify tissue inflammatory response to dental plaque. Fluctuation in immune-inflammatory response from adolescence to adulthood to senescence is a matter of concern while planning treatment for periodontitis. A paucity of data regarding the effect of age on treatment outcomes is observed in current literature. The need exists for analyzing and exploring this aspect further for an insight into better patient care.

Age as a parameter can affect treatment outcomes at molecular bone level. Dynamics of bone metabolism through biomarkers, explored during post-surgical intervention, in different age groups may help in custom-tailoring of treatment plans. It can form a viable basis for supportive periodontal therapy recall schedules and treatment needs.

A bimolecular system of RANKL and osteoprotegrin (OPG) is decisive regulator of osteoclastic bone resorption under physiological and pathological conditions. Available literature shows dearth of evidence regarding effect of periodontal surgery on the levels of RANKL and OPG in periodontitis patients.

Age has been significantly understudied in the field of periodontology. The present study aims to evaluate the impact of age (non-modifiable risk factor) by monitoring clinical and osteoclastogenic bone biomarker (OPG & RANKL) changes in periodontal diseases following periodontal surgical therapy.

Materials and Methods

Study design and Participants

Study was designed as an interventional, prospective, longitudinal trial with physically healthy periodontitis patients visiting Out-patient Department of Periodontology from January 2017 to March 2020.

The patient selected were healthy, previously untreated patients of moderate-severe (clinical attachment loss 3-6 mm), generalized (>30% of the sites involved) chronic periodontitis with a slow or moderate rate of progression, fulfilling the criteria for generalized chronic periodontitis by Armitage GC (1999) or generalized, Stage II/ III Grade A/B periodontitis based on Papapanou *et al*. (2018) Criteria for Periodontitis. Study inclusion was based on good oral hygiene maintenance and optimal compliance by the patient following Phase I therapy.

Patients excluded were (i.) medically compromised (diabetes, rheumatic fever, liver or kidney disease, neurological deficiencies, immunological diseases or cancer) (ii.) under any therapeutic regimen with the ability to alter soft tissue or bone healing (iii.) history of medication/ periodontal treatment/ surgery in previous six months preceding to enrollment (iv.) patients with grade modifier like smoking/ tobacco chewing; pregnant or lactating women.

Ethical clearance (reference code: 89th ECM II B Thesis/P87) was obtained from the Institutional Ethics Committee. The study was conducted under the Declaration of Helsinki of the World Medical Association (2008). Written informed consent was taken from all participants meeting inclusion criterion.

Study groups

Sample size of 45 patients (15 patients per group) was calculated based upon the deviation in RANKL/OPG ratio preoperatively and postoperatively in the study by Dereka *et al.* (2010). Study groups were divided according to age:

» Group 1: Diagnosed patients of periodontitis in the age group of 16 -19yrs (Youth).
» Group 2: Diagnosed patients of periodontitis in the age group of 25 -45yrs (Adult).
» Group 3: Diagnosed patients of periodontitis in the age group of >60yrs (Old).

One quadrant from each enrolled patient was selected for surgery.

Clinical examinations

Clinical parameters were evaluated by a single investigator (PG) at every recall visit. Calibration training was done on 10 volunteers on consecutive days. All recordings were repeated until an acceptable consistency was attained, which was determined by an intra-class correlation coefficient of 0.80.

Plaque Index (PI; Silness and Loe, 1964), Gingival Index (GI; Loe and Silness, 1963), Bleeding on Probing (BoP), Pocket Probing Depth (PPD), Clinical Attachment Level (CAL) were recorded and documented at baseline, 12 weeks and 24 weeks post-surgery by the same operator. Measurements were recorded at six sites per tooth (mesiobuccal, distobuccal, mid-buccal, mesiolingual/palatal, distolingual/palatal, mid-lingual/palatal) using University of North Carolina (UNC-15; Hu-Friedy, Chicago, IL, USA) periodontal probe through the groove cut in a customized acrylic stent that served as a fixed reference point.

Sample collection and Biochemical analysis

One tooth from surgical quadrant with deepest PPD was selected as test tooth for recording and sampling. Pooled GCF was taken from test tooth by absorbent paper points (Sure-Endo, size #20, 6% taper) before clinical measurements at baseline and repeated at 24th postoperative week. Test site was air-dried post removal of supragingival plaque. Site isolation was done through absorbent cotton rolls during GCF collection. Paper points were gently inserted inside pocket till slight resistance felt and held in-situ for 30 seconds. Blood/saliva contaminated paper points were discarded. Immediate transfer was done in sterilized micro-centrifuge tube (Eppendorf) containing 500 µl of phosphate buffer saline (PBS) at 40°C and stored at -80°C until assayed.

Prepared GCF samples centrifuged at 1000g for 20 minutes at 40°C were evaluated for identification and quantification of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegrin (OPG) using enzyme linked immunosorbent assay (ELISA) following manufacturer's protocol. Biotin double antibody sandwich technology was used for ELISA reaction to assess RANKL (GenAsia, Catalogue No.- GA-E0637HM, Shanghai, China) and OPG (GenAsia, Catalogue No.- GA-EI1575HM, Shanghai, China). Reaction was stopped by adding 50µl Stop Solution to each well (marked by immediate change in color from blue to yellow). Within ten minutes of adding the stop solution, taking blank well as zero for reference, absorbance optical density (OD) of each well was measured sequentially under 450nm wavelength. According to standards concentrations and corresponding OD values, the linear regression equation of the standard curve was calculated. Then the concentration of the corresponding sample was evaluated following the respective OD value using statistical software in the Department of Health research-Multi Research Unit of the University.

Surgical protocol

All patients enrolled in the study received thorough phase–I therapy and were given detailed oral hygiene instructions. Patients not responding to non-surgical therapy and maintaining adequate oral hygiene for 3 months post-non-surgical periodontal therapy were enrolled for surgical pocket therapy. Surgical technique was standardized as open flap debridement without resective/regenerative treatment for all patients. Surgical quadrant was anesthetized with solution of 2% lignocaine hydrochloride and adrenaline (1:80,000).

Intra-crevicular or internal bevel incisions were given buccally and lingually extending at least one tooth mesial and one tooth distal to the test tooth. The full-thickness/mucoperiosteal flap was reflected both buccally and lingually using the periosteal elevator to allow for complete debridement with minimal soft tissue trauma. The exposed area and root surfaces were carefully debrided and root planing was done using site-specific Gracey curettes (Hu-Friedy, Chicago, IL, USA). The surgical site was secured using interrupted sutures with 4-0 braided black silk sutures (Mersilk, Ethicon, UK) and periodontal dressing (Coe-pak, Coe Laboratories Inc, Chicago, USA).

Similar post-surgical instructions were given to all patients irrespective of the groups. Periodontal dressing and sutures were removed after ten days. Scheduled follow-up appointments were made at 4, 12, and 24 weeks postoperatively. Patients were re-evaluated clinically at 12 weeks and 24 weeks post-surgery, whereas GCF collection was done at 24 weeks follow-up.

Statistical analysis

Results were analyzed using descriptive statistics. Discrete (categorical) data were summarized as proportions and percentages (%) and quantitative data were summarized as mean ± SD. Changes in clinical (PPD, CAL) and biochemical parameters were assessed using ANOVA for repeated measures at different time points. A comparison between changes at different time intervals in a group was done through Student's paired t-test. Bi-comparison of clinical and biochemical markers between various group pairs was done using the Tukey HSD test and expressed as mean difference ± SD. Data of PI and GI were represented as box and whisker plots. A two-sided (α=2) $p<0.05$ was considered statistically significant.

Results

Patients selected for study were 108, out of which 92 (Group-I: 23; Group-II: 40; Group-III: 29) patients completed phase 1 therapy (Figure 1). Sixteen patients did not return after initial therapy or declined to participate. Twenty-eight patients (Group-I: 02; Group-II: 16; Group-III: 10) declined surgery and were put on non-surgical maintenance protocol. The total patient selected in each group was fifteen and the remaining patient (Group-I: 06; Group-II: 09; Group-III: 04) maintained a reserve pool with baseline data collected and samples were taken to counter dropouts if any at 24 weeks. Patients were randomly selected through computer-generated random numbers and all selected patients completed the study. Different randomization numbers were drawn for males and females and groups were well-matched for gender distribution with the proportion of Female: Male as 46.7% (7):53.3% (8) in all three groups.

Groups were divided based on age; the mean age of Group-1 (Youth) was 18.13±1.25 years, Group-2 (Adult) was 32.4±1.22 years, and Group -3 (Old) was 61.40±1.24 years.

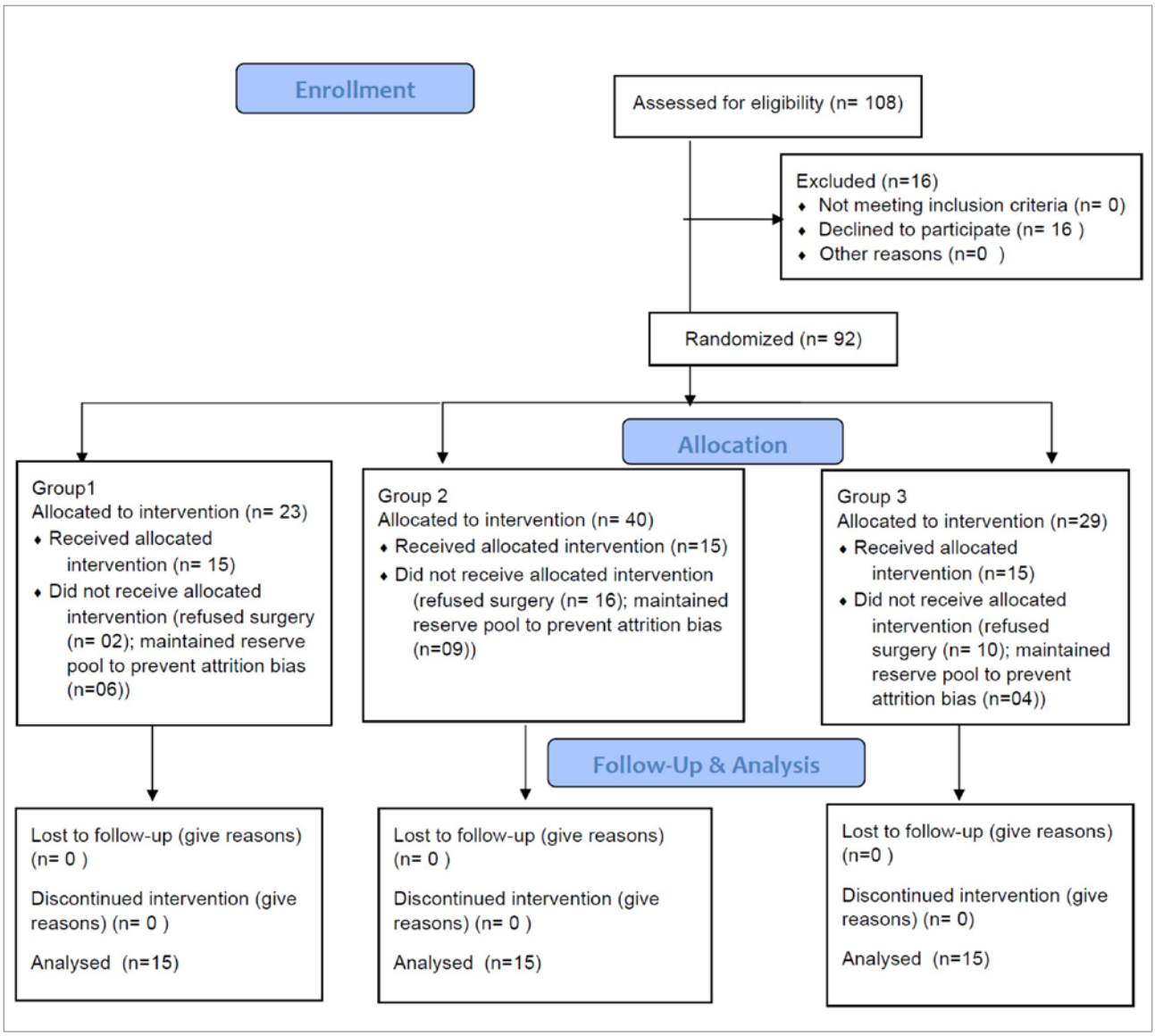

Figure 1. Study Flow chart.

One test tooth per subject was taken for analysis and pooled sample collection from all six sites. Comparisons of all age groups showed that plaque index and gingival index scores for test tooth were reduced in all three groups from baseline to 24 weeks post-surgery (Table 1). No statistically significant difference was observed between groups at any given time-period calculated on mean±SD values. The PI and GI score distribution with the median at different time points is depicted through box and whisker plots in Figure 2.

Intragroup comparison of Bleeding on probing (BoP) revealed significant differences from baseline to 24 weeks in the youth and adult group (Table 1). However, in the old group, significant differences were observed from baseline to 12 weeks ($p<0.001$), baseline to 24 weeks ($p<0.001$) but insignificant from 12 weeks to 24 weeks ($p=0.217$).

The mean PPD for test tooth was a minimum of 6.51±0.44 in Youth and a maximum; 6.64±0.56 in Old-group at baseline with no significant statistical difference ($p=0.774$) (Figure 2). Bi-comparison of PPD values observed insignificant differences between all the group pairs at baseline and each follow-up ($p>0.05$). Intra-group comparison showed significant differences from baseline-12 week ($p<0.001$), baseline-24 week ($p<0.001$) but insignificant from 12-24 week ($p=0.173$; $p=0.534$; $p=0.473$) in Youth, Adult and Old respectively.

Similar results were observed for the mean CAL of test tooth (Table 1; Figure 3). Intragroup comparison of CAL revealed an insignificant difference from 12-24 weeks in Youth ($p=0.791$) and Adult ($p=0.391$) but a significant difference in Old ($p=0.047$). At baseline, the mean RANKL and OPG score for GCF sample from test tooth were minimum 442.73±7.81

Table 1. Intragroup Comparisons of Clinical & Biomarker Values from Baseline to 24th Week Post-surgery in Youth, Adult and Old Age groups.

BL - 24th week comparison	Youth (Group 1)			Adult (Group 2)			Old (Group 3)		
	Mean Diff±SD	*t*	*p*	*Mean Diff±SD*	*t*	*p*	*Mean Diff±SD*	*t*	*p*
PI	1.68±0.29	22.19	**<0.001**	1.77±0.43	16.01	**<0.001**	1.90±0.40	18.51	**<0.001**
GI	0.71±0.26	10.55	**<0.001**	0.69±0.18	14.64	**<0.001**	0.72±0.30	9.12	**<0.001**
BOP%	65.86±6.15	41.49	**<0.001**	66.98±10.27	25.27	**<0.001**	66.84±11.17	23.18	**<0.001**
PPD	2.71±0.66	15.86	**<0.001**	2.75±0.68	15.57	**<0.001**	2.79±0.46	23.67	**<0.001**
CAL	1.44±0.67	8.25	**<0.001**	1.68±1.03	6.33	**<0.001**	1.51±0.95	6.15	**<0.001**
RANKL	-5.20±8.50	-2.37	**0.033**	-5.13±8.42	-2.36	**0.033**	-4.60±6.74	-2.64	**0.019**
OPG	-0.73±1.28	-2.22	**0.044**	-0.73±1.39	-2.05	0.060	-0.47±1.06	-1.70	0.110
RANKL/OPG	0.00±0.04	-0.25	0.809	0.00±0.05	-0.28	0.784	-0.02±0.04	-1.46	0.167

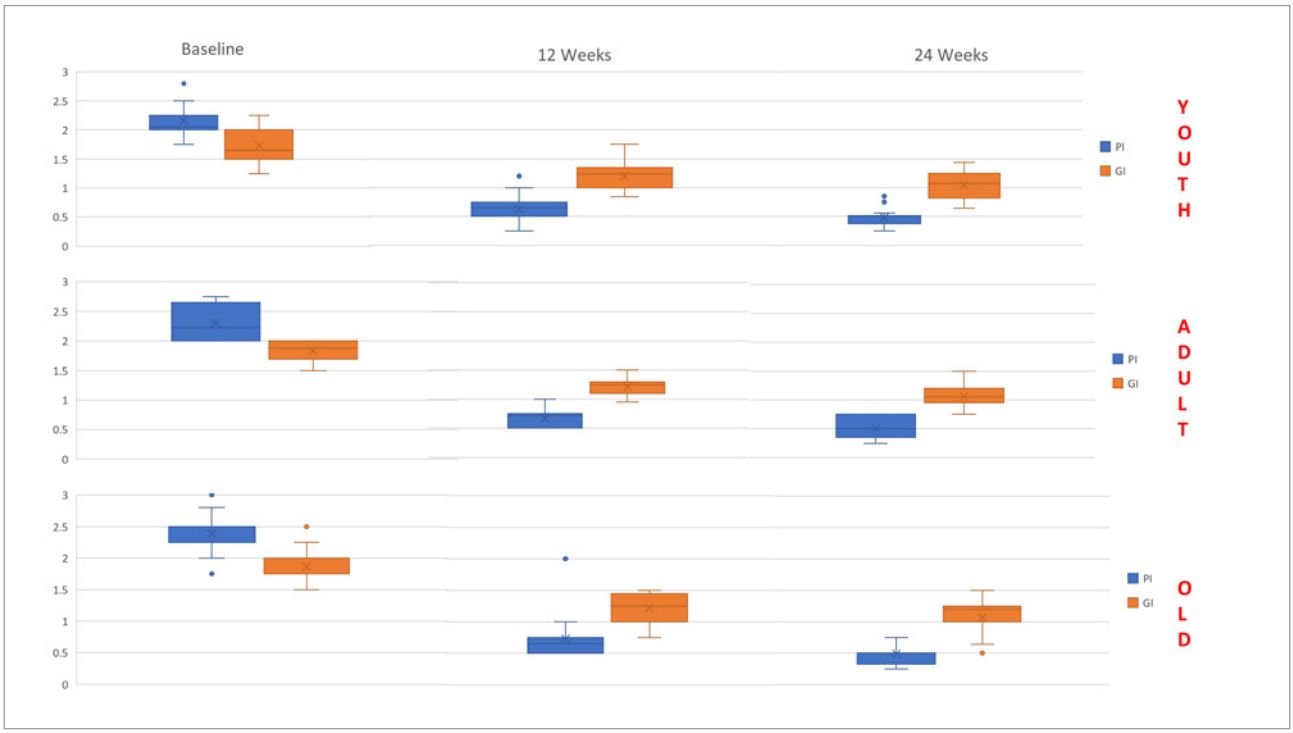

Figure 2. Box and Whisker plot of change in Plaque Index and Gingival Index at different time points for Youth, Adult and Old patients following surgical periodontal therapy. Boxes are representative of inter-quartile range (25th -75th percentile), and whiskers indicate the 5th and 95th percentiles for unadjusted data. Median is represented by horizontal line at the middle of the box. PI: Plaque Index; GI: Gingival Index.

(RANKL); 65.67±1.23 (OPG) in Youth and maximum 448.67±6.78 (RANKL); 66.47±1.06 (OPG) in Old-Group. No significant difference was observed in mean RANKL and mean OPG among the three groups at baseline (p=0.093; p=0.158) or 24th-week post-surgery sample (p=0.364; p=0.586) (Table 1; Figure 4).

The baseline means RANKL/OPG ratio was maximum (6.75±0.03) in the Old group. No significant difference was observed in mean RANKL/OPG among the three groups at baseline (p=0.567).

The post-surgical 24th-week sample revealed minimum values for mean RANKL/OPG ratio for Youth (6.74±0.04) and maximum for the old group (6.77±0.06). However, no statistically significant difference was observed among the three groups in the 24th week (p=0.277) (Table 1, Figure 3).

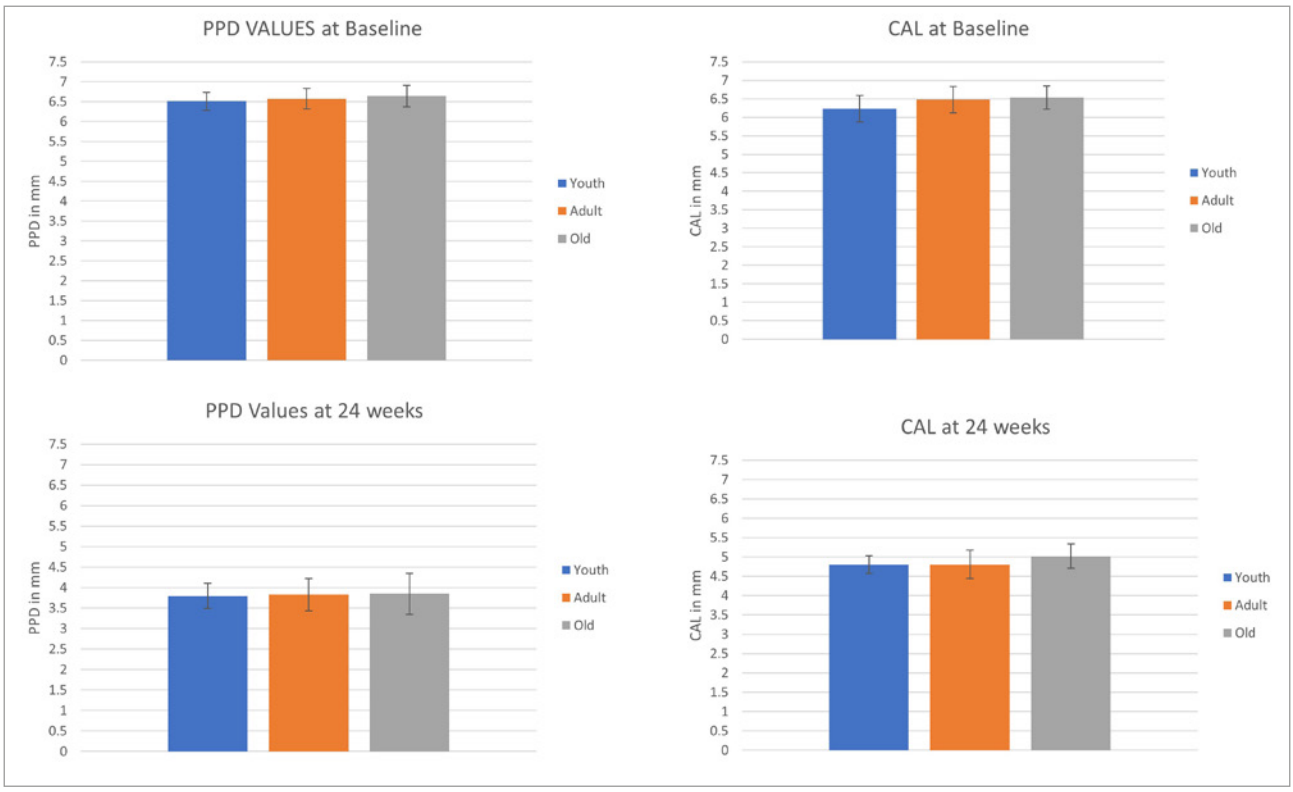

Figure 3. Mean±SD of PPD & CAL scores with confidence interval at baseline and 24 weeks post-surgery for youth, adult and old age groups. PPD: Probing pocket depth; CAL: Clinical attachment level.

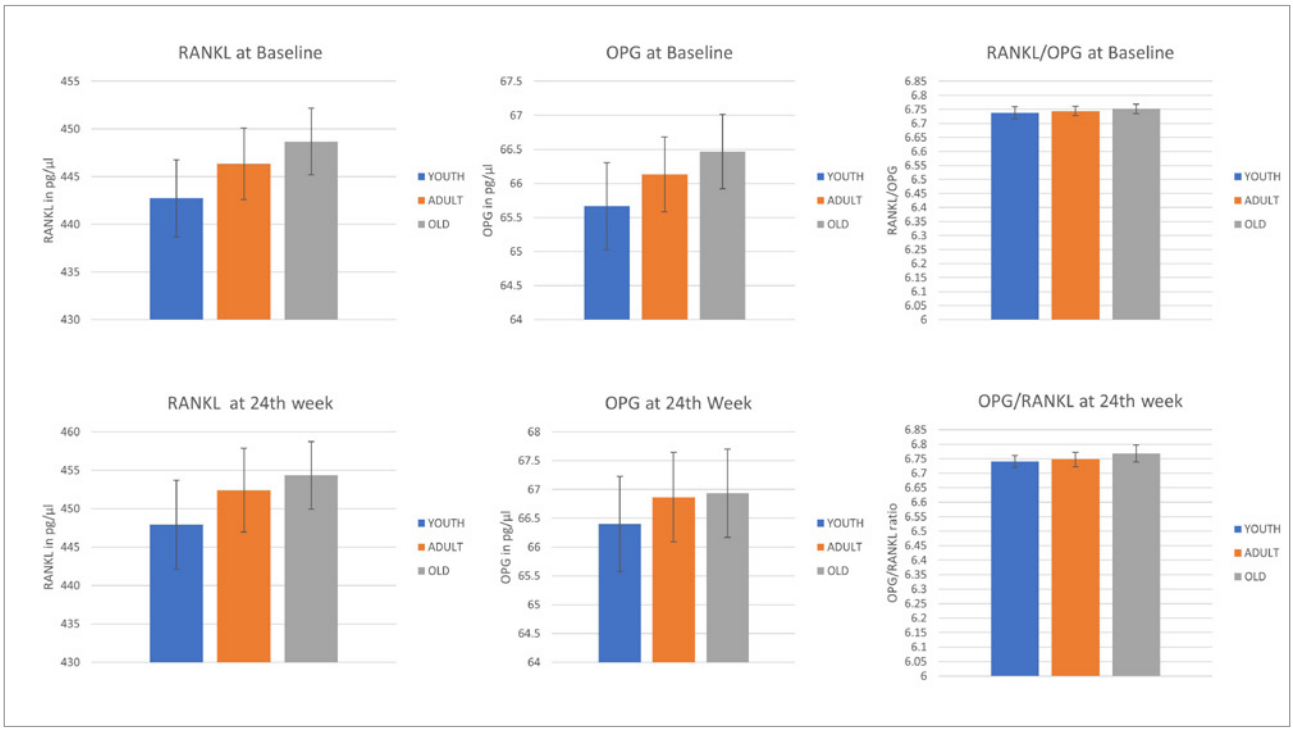

Figure 4. Mean±SD of RANKL, OPG and RANKL/OPG ratio with confidence interval at baseline and 24 weeks post-surgery for youth, adult and old age groups. RANKL: Receptor activator of nuclear kappa B receptor; OPG: osteoprotegrin.

Table 2. Comparisons of Clinical & Biomarker Values at various time points in Youth, Adult and Old Age groups.

Variable	Time point	Treatment groups			f value	p value
		Group1 (Youth)	Group 2 (Adult)	Group3 (Old)		
PPD	Baseline	6.51±0.44	6.57±0.50	6.64±0.53	0.26	0.774
	12th week	3.80±0.59	3.85±0.77	3.97±0.90	0.19	0.825
	24th week	3.80±0.59	3.83±0.76	3.85±0.42	0.03	0.974
CAL	Baseline	6.24±0.69	6.48±0.69	6.53±0.61	0.85	0.434
	12th week	4.81±0.43	4.92±0.85	5.10±0.58	0.75	0.479
	24th week	4.80±0.45	4.80±0.71	5.02±0.60	0.67	0.517
RANKL	Baseline	6.24±0.69	6.48±0.69	6.53±0.61	0.85	0.434
	24th week	4.80±0.45	4.80±0.71	5.02±0.60	0.67	0.517
OPG	Baseline	65.67±1.23	66.13±1.06	66.47±1.06	1.93	0.158
	24th week	66.40±1.59	66.87±1.51	66.93±1.49	0.54	0.586
RANKL/OPG	Baseline	6.74±0.04	6.74±0.03	6.75±0.03	0.58	0.567
	24th week	6.74±0.04	6.75±0.05	6.77±0.06	1.32	0.277

Discussion

Literature is full of studies on the effect of modifiable risk factors on treatment outcomes but there is a paucity of data regarding the effect of non-modifiable risk factors like age.

Age criterion used in this study was the young adolescent group (16- 19 years), stable adult group (25-45 years), and old geriatric group (> 60 years). Youth, as defined by the Macmillan dictionary, was the time between childhood and adulthood (maturity). The age range for youth is not consistent between cultures and regions (Tyyska, 2005). In the pretext of this study, youth age selection was based on pubertal teenage to identify patients in the growth spurt and hormonal fluctuation phase. United Nations considers youth till the age of 24 years, so the Adult age group was selected from 25-45 years to prevent overlap and to include a physiologically and osteologically stable age group as the positive control. Old age is the chronological age point where active participation of an individual in the society is limited or no longer possible (Gorman, 1999). The old age of >60 years was equivalent to retirement age in most institutions of our country and across the world and can be safely considered as the beginning of old age.

Standardized measurements and clinical/surgical protocols were followed for patients in all groups. Enrolled study participants showed good compliance and uneventful postoperative healing. Oral hygiene maintenance assessed through PI was comparable and good in all three age groups (Table 1, Figure 2). A similar study having age groups (26-79years) corresponding to Adult and Old of this study found comparable results with consistently reduced values for PI from 1.6 at baseline to 0.41 immediately after surgery and 0.35 at one-year follow-up on seventy patients (Lindhe and Nyman, 1975). Another study derived the incidence of periodontitis from the age-specific prevalence and found that age does not affect disease progression if good oral hygiene was maintained (Al-Nasser *et al*., 2020). Present study results emphasize that patient's frequent recall, motivation regarding oral hygiene measures, removal of plaque retentive areas during surgery, and maintenance of good plaque scores at all time points are more important considerations in healing outcomes than patient's age. Evaluation was done at 12 weeks and 24 weeks post-surgery in the present study. The influence of periodontal healing in the first 12 weeks can also attribute to clinical improvements irrespective of age.

Study observations are in concordance to that of Lindhe and Nyman (1975); Westfelt *et al*. (1983) regarding mean GI at baseline to a consistently low value at each recall visits post-surgery. Six months post-surgery results in group 1 (Youth) and group 3 (Old) were comparable to another study done by Lindhe *et al*. (1985) illustrating the significant improvement in mean GI from 1.7 at baseline to 0.3 and 0.5 respectively at 24 weeks follow-up.

CAL is an indicator of cumulative tissue destruction, including past periodontal disease, while PPD is an indicator of current disease status (Mdala *et al*., 2014). Periodontitis is a progressive disease with an increase in CAL loss in individuals aged 60-69 years. (Rheu *et al*., 2011) The present study found PPD and CAL scores to be maximum for the Old group and minimum for Youth with no statistical difference. PPD reduction and CAL gain in the present study was comparable to study by Lindhe *et al*. (1985), who in a sample of sixty-two patients (age groups <40 yrs, 40-49yrs, and >49 yrs) observed similar pocket depth reduction and gain in CAL of deeper pockets in all three

age groups at 24 weeks follow-up. Comparable results were observed for adult and old age groups in another study which showed progressive reduction in the mean PPD from 5.7 mm to <3mm at each follow-up after a modified reverse bevel flap surgery in a group of seventy patients with an age range of 26-79yrs (Lindhe and Nyman, 1975). All three groups showed no statistical difference in results with Youth (Group-1) showing the most stable pocket depth after 12 weeks as compared to other groups. Similar trend was observed in a study on 229 patients (20-79yrs) with significant pocket depth reduction at 12 and 24 weeks (Mdala *et al*., 2012). However, the study is in contrast with regards to CAL where attachment loss was observed following surgery.

Biomarkers of bone metabolism show significant variation with age with levels reaching a peak during a pubertal growth spurt, followed by a rapid decline to adult levels (Weaver *et al*., 1997; Rauchenzauner *et al*., 2007).

Surgical therapy in periodontitis involves alveolar bone exposure and healing dynamics of bone coupling. RANKL/RANK regulates osteoclast signaling and bone remodeling. OPG and RANK have a competitive binding to RANKL, with OPG preventing excessive resorption. The relative concentration of OPG and RANKL is considered a major determinant of periodontal disease or health. RANKL/OPG plays a central role in this coupling balance of bone metabolism (Vega *et al*., 2007). They may be considered as reliable biomarkers detailing the state of periodontal disease. (Buduneli and Kinane, 2011) The present study used RANKL and OPG as biomarkers for assessing the changes in bone healing mechanisms following surgical therapy in different age groups.

In youth, some studies have shown an increased value of OPG with age (Gajewska *et al*., 2006), some observed an inverse relationship between the two (Buzi *et al*., 2004), while others found no correlation between OPG and age (Ozkaya *et al*., 2007; Wasilewska *et al*., 2011). RANKL showed a significant positive association with age (Kerschan-Schindl *et al*., 2008; Wasilewska *et al*., 2011). RANKL/OPG ratio also showed a positive correlation with age (Ali *et al*., 2019). A recent cohort study on 300 children in the age range of 1-21years observed a significant difference in RANKL and RANKL/OPG levels by age (decreased with age except for 11-15yrs) while OPG showed no relation with age (Ali *et al*., 2019). Young age groups (< 30yrs) demonstrated low variability for OPG value while the old group (> 60yrs in female and > 70yrs in the male) showed greater variability (Yano *et al*., 1999; Szulc *et al*., 2001). Though effect of Age on RANKL and OPG during healthy state has been studied, the paucity of evidence correlating RANKL, OPG, and age in the diseased periodontium and post-therapy exists.

Gingival crevicular fluid (GCF) is an altered serum transudate, changing its nature to inflammatory exudate when signs of periodontal inflammation become clinically evident (Sorsa *et al*., 2000). In contrast to saliva, GCF is secreted subgingivally in response to periodontopathogenic stimulation; therefore, it acts as an exclusive window to quantitatively and qualitatively assess the magnitude of the bacterial challenge as well as the host response against them to regain homeostasis (Golub *et al*., 1997). Many bone turnover-related biomarkers have been detected in GCF, (Lamster & Ahlo, 2007) emanating as possible markers of periodontal disease activity (Buduneli and Kinane, 2011), thus providing a judicious ground for investigating RANKL and OPG in this study. RANKL/OPG ratio, which is an established chief regulator of osteoclastogenesis, can be used as a molecular diagnostic marker of periodontal destruction to detect changes across various age groups.

Intergroup comparison showed no significant difference in mean RANKL value among the three groups at baseline and at 24 weeks post-surgery with minimum (442.73±7.81; 447.93±10.81) for Youth and maximum (448.67±6.78; 453.27±9.21) for Old age groups. Larger sample size is required to make a conclusive statement about the effect of this minuscule difference. Study results are comparable to study by Bostanci *et al.* (2011) which observed mean RANKL value to be 433 ±269 pg/ml at baseline in periodontitis patients irrespective of age. Another study observed insignificant change in mean RANKL value at 12 weeks following surgical therapy indicating no effect of periodontal surgery on RANKL in spite of improved clinical outcomes (Ilarslan *et al*., 2016). In our study, significant difference in RANKL value from baseline to 24 weeks was observed in all the three groups inferring to a molecular mechanism of active bone resorption leading to increased risk of disease relapse at the treated site irrespective of age.

Youth showed significant difference in mean OPG value while Adult and Old age-groups showed insignificant difference. Study results are in contrast to a study in 20 periodontitis patients (35-55 years) which found significant increase in mean OPG levels in GCF from baseline to 24 weeks post OFD (Hassan *et al*., 2015). Present study depicts that decrease in inflammation might not necessarily lead to OPG increase. Miscellaneous mechanisms regulate bone remodeling biomarkers. Disparity in sampling techniques, sensitivity/specificity of the immunoassays, and intraindividual differences between study populations and sample size could be accredited to these variations in results.

Nearly constant value in mean RANKL/OPG ratio in present study even after treatment is in accordance to other studies following non-surgical periodontal therapy (Bostanci *et al*., 2011) and surgical therapy (Ilarslan *et al*., 2016).

Dereka *et al*. (2010) observed slight increase in RANKL/OPG expression ratio in patients of periodontitis following non-surgical periodontal treatment. Accumulated evidences from the previous and present studies indicate that clinically successful periodontal therapy may not necessarily reduce this ratio and success of periodontal therapy cannot be judged by RANKL/OPG ratio. This does not mean the failure of RANKL/OPG ratio to act as a potential diagnostic marker for periodontitis as it could still indicate sites with disease recurrence at molecular level. Results of present study indicate that as biomarkers RANKL/OPG lack sensitivity in assessing the outcomes of periodontal therapy.

Most of the available studies compared RANKL and OPG among periodontitis patients with healthy controls. To the best of our knowledge except for the study by Hassan *et al.* (2015) and Ilarslan *et al.* (2016), no other study has investigated RANKL and OPG following surgical therapy. RANKL/OPG ratio can be used as a risk indicator for periodontal disease progression and /or predictor of ongoing disease activity but for that, this ratio needs to be defined more accurately. Lack of defined values for RANK, RANKL and OPG makes it difficult to assess accurately a healthy periodontium from diseased site. Various protocols need to be standardized for proper utilization of RANKL-OPG system in clinical periodontology. These include global standardized technique for sample collection and detection assays.

Within its limitations, this study showed no significant difference among three groups on clinical parameters and on RANKL, OPG and RANKL/OPG ratio.

References

Akhtar Ali S, Kang H, Olney R, *et al*. Quantifying RANKL and OPG levels in healthy children: A large cross-sectional analysis. *Bone* 2019;**127**:215-219.

Al-Nasser L, Lamster IB. Prevention and management of periodontal diseases and dental caries in the older adults. *Periodontol 2000*. 2020;**84**(1):69-83.

Belibasakis GN. Microbiological changes of the ageing oral cavity. *Arch Oral Biol* 2018;**96**:230-2.

Bostanci N, Saygan B, Emingil G, Atilla G and Belibasakis GN. Effect of periodontal treatment on receptor activator of NF-κB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid. *Journal of Clinical Periodontology* 2011;**38**:428-433.

Brunetti G, Colucci S and Pignataro P. T Cells support osteoclastogenesis in an in vitro model derived from human periodontitis patients. *Journal of Periodonto*logy 2005;**76**:1675-80.

Buduneli N and Kinane DF. Hostderived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *Journal of Clinical Periodontology* 2011;**38**:S85-S105.

Buzi F, Maccarinelli G and Guaragni B. Serum osteoprotegerin and receptor activator of nuclear factors κB (RANKL) concentrations in normal children and in children with pubertal precocity, Turner's syndrome and rheumatoid arthritis. *Clinical Endocrinology (Oxford)* 2004;**60**:87-91.

Dereka XE, Markopoulou CE, Fanourakis G, Tseleni-Balafouta S and Vrotsos IA. RANKL and OPG mRNA level after non-surgical periodontal treatment. *Inflammation* 2010;**33**:200-206.

Franceschi C. An evolutionary perspective on immunosenescence. *Annals of New York Academy of Sciences* 2000;**908**:244-254.

Gajewska J, Ambroszkiewicz J and Laskowska-Klita T. Osteoprotegerin and C-telopeptide of collagen in Polish healthy children and adolescents. *Advances in Medical Sciences* 2006;**51**:269-272.

Golub LM, Lee HM, Greenwald RA, *et al*. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflammation Research: Official Journal of the European Histamine Research Society* 1997;**46**:310-319.

Gorman M. *Development and the Rights of Older People*. In: Randel J *et al*. (Eds): *The ageing and development report:poverty, independence and the world's older people.* London, Earthscan Publications Ltd.,1999:3-21.

Hassan SHS, El-Refai MI, Ghallab NA, Kasem RF and Shaker OG. Effect of periodontal surgery on osteoprotegerin levels in gingival crevicular fluid, saliva, and gingival tissues of chronic periodontitis patients. *Disease Markers.* 2015; 341259.

Ilarslan YD, Erman B, Karabulut E, Tezcan I and Berker E. Effect of periodontal surgery on the Osteoimmunological mediators in patients with chronic and Aggressive periodontitis. *Clinical Dentistry and Research* 2016;**40**:49-59.

Kayal RA. The role of osteoimmunology in periodontal disease. *Biomedical Research International* 2013;2013(639368).

Kerschan-Schindl K, Wendlova J, Kudlacek S. Serum levels of receptor activator of nuclear factor kappaB ligand (RANKL) in healthy women and men. *Experimental and Clinical Endocrinology and Diabetes* 2008;**116**:491-495.

Kornman KS, Duff GW. Personalized medicine: will dentistry ride the wave or watch from the beach? *Journal of Dental Research* 2012;**91**:8S-11S.

Lamster IB and Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Annals of the New York Academy of Sciences* 2007; 1098:216-229.

Lindhe J and Nyman S. The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease. *Journal of Clinical Periodonlology* 1975;**1**:67-79.

Lindhe J, Socransky S, Nyman S, Westfelt E and Haffajee A. Effect of age on healing following periodontal therapy. *Journal of Clinical Periodonlology* 1985;**12**:774-787.

Loe H and Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* 1963;**21**:533-551.

Mdala I, Haffajee AD, Socransky SS, *et al*. Multilevel analysis of clinical parameters in chronic periodontitis after root planing/scaling, surgery, and systemic and local antibiotics: 2-year results. *Journal of Oral Microbiology* 2012;**4**:17535.

Mdala I, Olsen I, Haffajee AD and Socransky SS. Thoresen M, de Blasio BF. Comparing clinical attachment level and pocket depth for predicting periodontal disease progression in healthy sites of patients with chronic periodontitis using multi-state-Markov models. *Journal of Clinical Periodonlology* 2014;**41**:837-845.

O'Connor J-LP, Milledge KL, O'Leary F, Cumming R, Eberhard J, Hirani V. Poor dietary intake of nutrients and food groups are associated with increased risk of periodontal disease among community-dwelling older adults: a systematic literature review. Nutr Rev 2020;**78**(2):175-88.

Ozkaya O, Buyan N and Bideci A. Osteoprotegerin and RANKL serum levels and their relationship with serum ghrelin in children with chronic renal failure and on dialysis. *Nephron Clin Pr* 2007;**105**:153-158.

Papapanou PN, Sanz M, Buduneli N, *et al*. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology* 2018;**45**:S162-S170.

Peters A, Delhey K, Nakagawa S, Aulsebrook A and Verhulst S. Immunosenescence in wild animals: meta-analysis and outlook. *Ecology Letters* 2019;**22**:1709-1722.

Rabiei M, Masoudi Rad H, Homaie Rad E, Ashourizadeh S. Dental status of the Iranian elderly: A systematic review and meta-analysis. *J Investig Clin Dent*. 2019;**10**(4):e12459.

Rauchenzauner M, Schmid A, Heinz-Erian P, Kapelari K, Falkensammer G and Griesmacher A. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *The Journal of Clinical Endocrinology and Metabolism* 2007;**92**:443-9.

Rheu G-B, Ji S, Ryu J-J, *et al*. Risk assessment for clinical attachment loss of periodontal tissue in Korean adults. *The Journal of Advanced Prosthodontics* 2011;**3**:25-32.

Silness J and Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* 1964;**22**:121-135.

Silva N, Abusleme L and Bravo D. Host response mechanisms in periodontal diseases. *Journal of Applied Oral Sciences* 2015;**23**:329-55.

Sorsa T, Gursoy UK, Nwhator S, *et al*. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. *Periodontol*ogy 2000 2016;**70**:142-163.

Szulc P, Hofbauer LC, Heufelder AE, Roth S and Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. *The Journal of Clinical Endocrinology and Metabolism* 2001;**86**:3162-3165.

Tyyska, V. Conceptualizing and theorizing youth: global perspectives. In: *Contemporary Youth Research: Local Expressions and Global Connections; London: Ashgate Books*; 2005:3.

Vega D, Maalouf NM and Sakhaee K. Clinical Review #: the role of receptor activator of nuclear factor-kappa B (RANK)/ RANK ligand/ osteoprotegerin: clinical implications. *Journal of Clinical Endocrinology and Metabolism* 2007;**92**:4514-4521.

Wasilewska A, Rybi-Szuminska AA and Zoch-Zwierz W. Serum Osteoprotegrin (OPG) and Receptor Activator of Nuclear Factor κB (RANKL) in Healthy Children and Adolescents. *Journal of Pediatric Endocrinology and Metabolism* 2011;**22**:1099-1104.

Weaver C, Peacock M, Martin B, McCabe G, Zhao J and Smith D. Quantification of biochemical markers of bone turnover by kinetic measures of bone formation and resorption in young healthy females. *Journal of Bone Mineral Research* 1997;**12**:1714-20.

Westfelt E, Nyman S, Lindhe S and Socransky S. Use of chlorhexidine as a plaque control measure following surgical treatment of periodontal disease. *Journal of Clinical Periodonlology* 1983;**10**:22-36.

Yano K, Tsuda E and Washida N. Immunological characterization of circulating osteoprotegerin/ osteoclastogenesis inhibiting factor: increased serum concentrations in postmenopausal women with osteoporosis. *Journal of Bone Mineral Research* 1999;**14**:518-27.