Research Article

Levels of Cytokine Expression and Fixed Orthodontic Treatment Timing between the Menopausal and Young Women

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ABSTRACT

Introduction: Menopause and the use of OCP bring about a lot of changes in the female biology. Estrogen is essential for the maintenance of the bone health in adulthood and its withdrawal attributes to the production of osteoclastogenic cytokines.

Objective: The first objective was to detect the influence of menopause and OCPs in GCF in women of various age groups during orthodontic treatment. Second was to determine the difference in orthodontic treatment timing in menopausal and young women.

Materials and Method: The GCF samples were collected from 48 healthy female individuals, age ranging from 22 to 56 years. There were four groups of women. First group consisted of 24 menopausal women, second group consisted of 24 young women. The other two groups consisted of 12 young women taking OCP and the other 12 young women not taking OCP. Cytokine analysis was done with the help of enzyme-linked immunosorbent assay. Treatment time to grossly align the mild crowding of the lower incisors was noted as the fixed orthodontic treatment time.

Result: The expression of RANKL and IL-17A was higher in menopausal women than the young women ($p<0.05$). Significantly higher expression of RANKL and IL-17A was observed in the young women without the OCP than the women using OCP. Treatment time was significantly shorter in the menopausal women than the young women. Treatment time significantly long in the women taking OCP ($p<0.05$) than in women not taking OCP.

Conclusion: The expression of higher and the comparatively lower levels of certain cytokines conclude the possible effect of female hormones while planning the treatment and consideration of age and time while rendering various types of orthodontic treatment modalities.

Keywords: Gingival crevicular fluid; menopause; oral contraceptive pill; orthodontic treatment time.

INTRODUCTION

Menopause causes substantial increase in the bone turnover and crucial imbalance in bone remodeling. In this state, there is increase in osteoclastogenesis that leads progressive trabecular bone mass loss.[1] Estrogen withdrawal attributes to the production of osteoclastogenic cytokines such as tumor necrosis factor (TNF), Interleukin (IL)-6, IL-1, Multinucleated- colony stimulating factor (M-CSF) and lymphoid cells. The increase productions of these factors are responsible for the differentiation of myeloid precursor cells into osteoclasts, which is turn are responsible for the bone resorption. Receptor activator of NFκB ligand (RANKL), IL-17A and IL-1 are responsible for sustaining the osteoclasts survival and RANK activity. Nuclear factor κB (NFκB) ligand, a member of the TNF family, expressed by the stromal cells is crucial for promoting osteoclast differentiation and activation. Excessive osteoclastogenic response is evident within five years of menopause.[1] Estrogen therapies have been seen to suppress the osteoclastogenic activity in the bone thus exhibiting its beneficial effects in preventing postmenopausal osteoporosis.[2] But for an Orthodontist this might create a possibility to endure a slight caution.

Menopausal age for Asian women in average is 51.5 years although there are factors like genetics, smoking, chemotherapy and ovarian surgeries that have a direct impact on its occurrence.[1] RANKL and IL-17 A are known important factors in the bone metabolism.[2,3] These affect actively in the process of bone remodeling during the Orthodontic tooth movement (OTM).[4-6] The effect of higher or lower expression of estrogen might as well have significant effects on the fixed orthodontic treatment time (FOTT) while treating the females of various age groups. This study focuses on the expression of different important cytokines responsible for the fixed OTM on patients under hormone therapy such as taking oral contraceptive pills (OCPs). In regard to Orthodontics, there are no researches so far to know about the effects and differences of menopause during the tooth movement and the treatment time required to finish a certain course of tooth movement. We tried to evaluate if there was any significant levels of cytokines and FOTT difference between the young female with/ without the OCP influence and the female after their menopause.

MATERIALS AND METHOD

A total of 48 healthy female individuals, age ranging from 22 to 56 years participated in the study. They were selected from the Department of Orthodontics, Wuhan Union Hospital, Wuhan, Hubei Province, People's Republic of China. All the patients underwent oral prophylaxis and the Plaque Index and Gingival index were recorded at the "0" and between "0.1 to 1.0" respectively in both the baseline, recall and at the day of sample collection. The participants were informed and the consent was signed before the process of sample collection. The inclusion criteria were the age between 22-56 years. Gingival sulcus within the 3 mm of probing depth was included in the study. The patients under any other medication other than the OCPs were not included in the study. The patients who needed interproximal stripping (IPS) for the correction of mild imbrication were included in the study. Whereas, the exclusion criteria included the probing depth more the 3 mm, severe gingivitis and periodontitis, diabetes, under antibiotics or anti-inflammatory within past 6 months and 30 days respectively of sample collection, carious lesions, bleeding disorders and pseudo pockets in the gingiva. Menopausal women with Estradiol levels <10 pg/mL were not included in the study.

The participants were divided into 4 groups. The first group included 24 women ranging from 51.5 to 55 years[8] of age and who had already experienced menopause for at least a year. The second group was of 24 young women ranging from 22 to 32 years of age. The second group was further divided into equal halves in the ratio of 1:1. This division of group constituted of 12 young women (third group) who were taking OCPs and the other half of 12 young women (the fourth group) not taking the medication. This ratio allocation was considerable, as it was a definitive representation of the statistical analysis. All the women were sent to the pathology laboratory for the determination of their estrogen levels. The estradiol levels for the Post-menopausal women ranges from 0-40pg/mL[8] but the menopausal women with Estradiol levels <10 pg/mL were not included in the study to exclude the biasness of the result while comparing with the young women without any OCP therapy. Women with estradiol levels <10 pg/mL, are prone to bone loss and osteoporosis as the levels of cytokines responsible for the osteoclastogenesis is very high. The estradiol levels of the young women included in the study was in the range of 96-436 pg/mL, the highest being in the pre-ovulatory phase and

the lowest at the follicular stage.[1] Above and below the ranges mentioned, were not included in the study.

The samples were collected within second orthodontic recall appointment, which is within 60 days of MBT bracket placement. The orthodontic patients are called for activation every 30th day and the treatment time to grossly align the malaligned lower front teeth has been noted. It has been considered the fixed OTM to grossly align the mild crowding of the teeth requiring Inter proximal stripping.

The gingival crevicular fluid (GCF) was collected by the Milipore filter membranes (pore size 0.22 mm; Milipore) from the distal medial and mesial sides of the lower central incisor at the initial alignment stage. All the orthodontic patients involved where the borderline cases with imbrication of (0-0.25 mm) and required IPS for the proper alignment of the dentition according to the Nance Carey's model analysis. The samples were collected and well preserved before the IPS, to avoid the chances of sample collection after the gingival trauma during the IPS, if any. The experimental tooth was cautiously rinsed with water, and the gingival area was isolated with cotton rolls and gently dried. The first Millipore polyvinylidene fluoride (PVDF) strip was very carefully inserted 1 mm into the sulcus and left in situ for 15 seconds. After removal of the first strip, a second PVDF strip was similarly inserted in the other two sites for 15 seconds. The strip contaminated with blood was discarded. After removal from the sulcus, each of the strips was placed in a Periotron 700100 (Oraflow) for GCF volumetric measurement. Within 60 minutes, proteins in the three strips were eluent in 500 isotonic phosphate buffered saline, pH=7.4, without detergent and protease inhibitors, and immediately frozen at –20°C until the day of the analysis. The number of freeze thaw cycles was kept to a minimum. Most of the proteins were reproducible and the recovery was satisfactory.

With the help of Sigma-Aldrich's 3-ethylbenathiazoline-6-sulfonic acid liquid substrate system for enzymelinked immunosorbent assay (ELISA) and the Human IL17A Mini ELISA Development Kit (900M84, PeproTech), all the samples were carefully analyzed for RANKL and IL-17A. All the strips were kept at the collar of the microcentrifuge tubes to elute all the GCF components completely and were centrifuged at 2,000 g for five minutes. After the strips' removal, the supernatant was divided into two aliquots for the correct determination of each biologic compound. The assays were carried out in accordance with the manufacturer's instructions and the levels of the biochemical compounds were reported as the optical density (OD) values.

To compare different variables in different sample groups, the one-way analysis of variance (ANOVA), using SPSS version 20 was done. The statistical significance of ($P<0.05$) was considered significant.

RESULT

The expression of RANKL and IL-17A was observed higher in menopausal women than the young women ($p<0.05$, Figure 1, 3). Significant higher expression of RANKL and IL-17A in the young females patients without the OCP than the females using OCP (Figure 2, 4). The treatment time was significantly faster in the menopausal women than the young women and the treatment time significantly long in the women under OCP ($p<0.05$, Figure 5, 6).

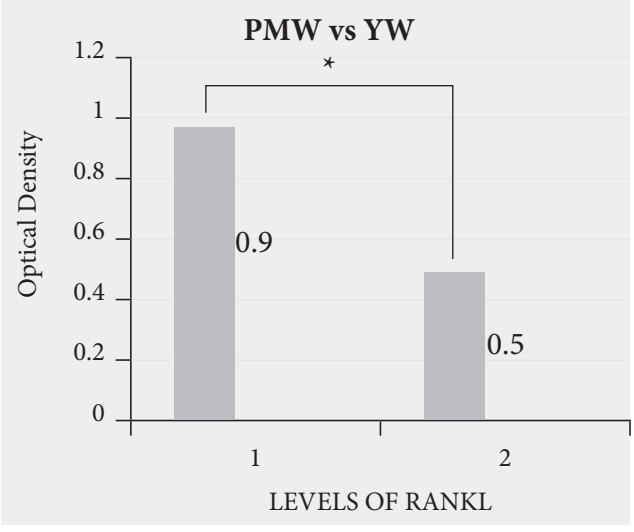

Figure 1: Significant difference in the expression of RANKL between post-menopausal women and young women.

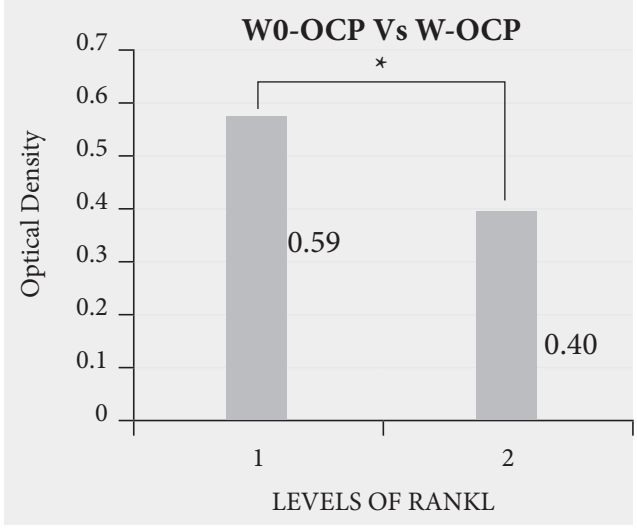

Figure 2: Significant difference in the expression of RANKL between young women without OCP and with OCP.

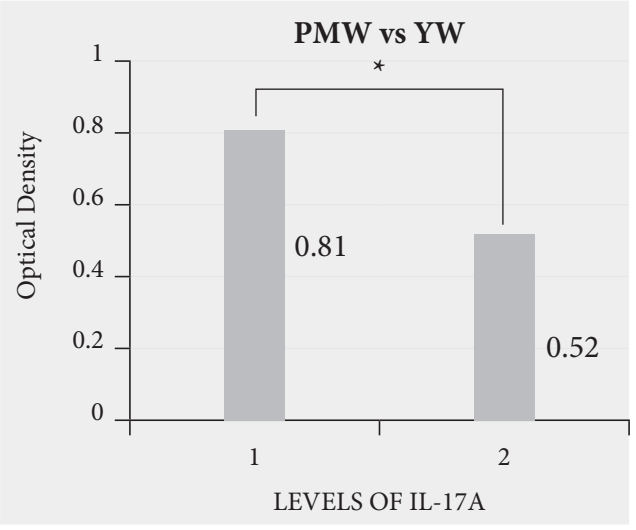

Figure 3: Significant difference in the expression of IL-17A between post-menopausal women and young women.

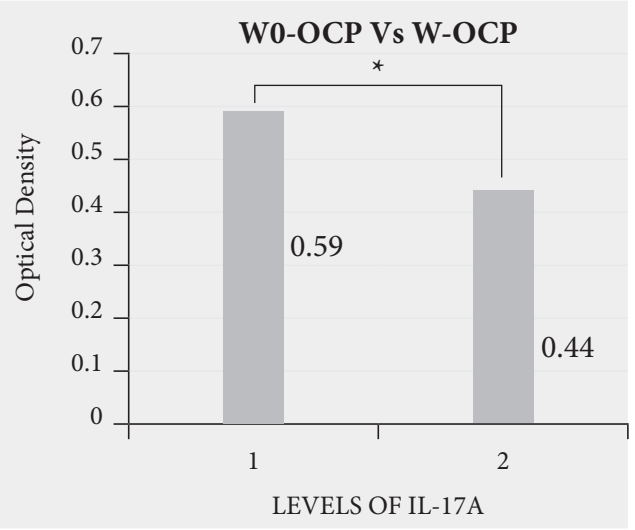

Figure 4: Significant difference in the expression of IL-17A between young women without OCP and with OCP.

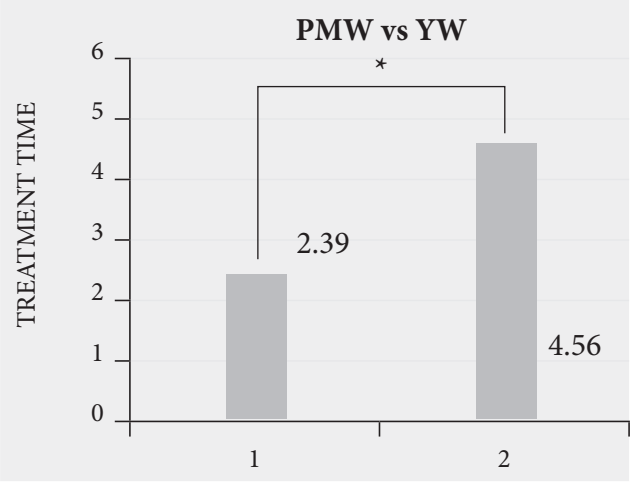

Figure 5: Significant fixed orthodontic time difference between postmenopausal women and young women undergoing orthodontic treatment.

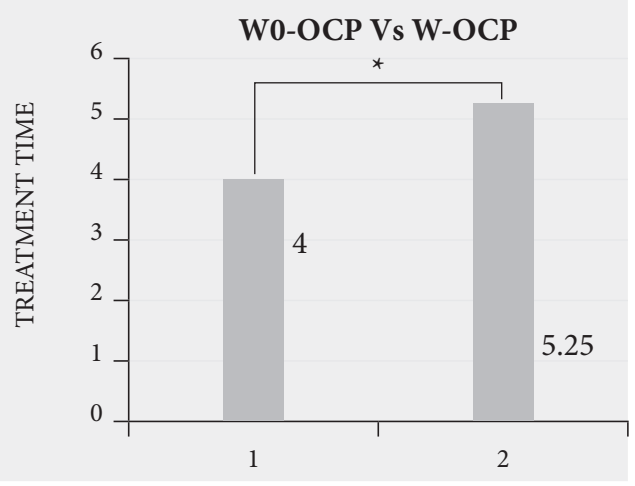

Figure 6: Significant fixed orthodontic time difference between women without OCP and young women with OCP undergoing orthodontic treatment.

DISCUSSION

In the present study, we have monitored the levels of cytokines essential for the bone remodeling during the fixed orthodontic treatment. The rise in the levels of these compounds within certain biological limits enhances the process of proper tooth movement whereas the unusually altered expression of these cytokines may lead to various consequences. We have tried to portray in this study that age, hormones may have significant impact on the FOTT especially in the females.

The results show that there is significant higher expression of RANKL and IL-17A in menopausal women than the young women. Authors direct this result to the estrogen deficiency in these women. Estrogen is known to suppress the osteoclastogenic cytokines responsible for the remodeling of bone. Menopausal women are deficit of estrogen and thus this fact advocates the significant high levels of the cytokines even in the elderly female Orthodontic Patients. The higher expression also emphasizes the strong process of ongoing inflammation, excessive osteoclastogenic effects which are the signs of lack of estrogen response to the bone turnover.

Not only estrogen, but also the hormone therapies as in the women taking OCPs women have shown significant lower expression of the cytokines than that of the young women not under the influence of OCP. OCP is the combination of female sex hormones, Estrogens and Progestin. This study is in line with other studies which hypothesize that estrogen might lessen the expressions of osteoclastogenesis inducing protein, fluctuation in serum marker bone turnover and acceleration of OTM after ovulation.[2,7,9] Along with the higher levels of protein expression is the significant shorter time taken to complete the initial stage of teeth alignment in the menopausal women

and the young women without the OCP effect. However, as this is study is a noble idea of research there aren't any studies to compare to.

Orthodontists are motivated towards providing excellent treatment to the patient. With these above results we know that the cytokines are estrogen dependent and so is FOTT. Not only the age or the medication but the mechanical forces induced by the brackets and the wires are directly responsible for bringing about the release of these cytokines, growth factors and colony stimulating factors that in turn bring about the bone remodeling. The results may be detrimental if precautionary measures are not practiced while treating menopausal women. The precautionary measures may be applying the slow and the continuous force within the optimum limits of 25gm/cm^2 If a prior investigation is made mandatory in these patients the devastating results can be avoided. Studies suggesting the interrelationship between the environmental factors (orthodontic treatment) and each patient's unique bone biology should be conducted, the treatment will be precise, treatment planning shall be refined and orthodontic force may be optimized.[10] Nevertheless, if not in all patients, a special investigation on menopausal women to eliminate the devastating effects of excessive mobility and poor gingival health should be carried out. Similarly, such investigations should also be carried out in the young women under the OCP influence. These tests may reduce the chances of delayed tooth movement and may help to manage and balance the orthodontic forces, thus making the orthodontist's work relatively easy.

CONCLUSION

The fact of higher levels of RANKL and IL-17A, shorter FOTT in menopausal women than the young women ($p<0.05$) may be attributed to the lower levels of estrogen in the older females. The young female under the OCP influence show significant lower levels of the cytokines and comparative longer FOTT. OCP is the supplement of female hormones Estrogens and Progestin.

This study indicates revising our History form, especially in the case of women. An addition of drug history, and years since menopause in older women will help us prescribe any further investigation and plan our treatment time accordingly. The result of this study may also influence our orthodontic consent form especially in regard to the FOTT. The patient should also be cautioned and informed accordingly.

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JNDA

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