



HISTOLOGICAL AND HORMONAL ANALYTICAL STUDIES FOR THE EFFECT OF ESTROGEN AND TESTOSTERONE REPLACEMENT ON TEMPOROMANDIBULAR JOINT OF GONADECTOMIZED RATS

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ABSTRACT

In this study, 42 male and female Sprague-Dawley rats were used and were divided into five groups including: the control group I involved 14 male and female rats, seven rats each, group II; involved 14 female rats subjected to ovariectomy, group III; involved 14 male rats subjected to testectomy. The group IV; involved 7 of the ovariectomized female rats were injected with estrogen replacement therapy while, the remaining 7 ovariectomized rats served as control. The group V; involved 7 of the testectomized male rats were injected with testosterone replacement therapy, while the other 7 testectomized rats served as control. The surgical interference of gonadectomy was performed under anesthesia and the ovaries of animals in group II were surgically accessed via lateral approach, while the testes of animals in group III were surgically accessed through the lower abdomen. The hormonal injection was performed after 50 days from surgery. The animals of group IV, were injected with estrogen depot intramuscularly with dose of 0.5 mg/kg body weight every 7 days for 4 weeks. The animals of group V, were injected with testosterone depot intramuscularly with dose of 20 mg/kg body weight every 7 days for 4 weeks. Development of lymphoid follicles in synovial membrane showed in ovariectomized rats and in testectomized rats showed proliferation of synovial villi with their invasion by numerous vascular sinusoids, fat cells or lipid-filled macrophages to synovial subintima and the posterior band of articular disc and not improvement in replacement therapy groups and the effect of decreased level of the testosterone on TMJ was more than the estrogen and there is not considerable improvement of TMJ was observed when the animals were treated with hormone replacement therapy.

INTRODUCTION

The prevalence, severity, and duration of the temporomandibular disorders (TMDs) were recorded to be greater in women than men. These TMDs were found to primarily affect the women during their reproductive years and this was reflected with the increased risk for the treatment of TMDs in women who administer the exogenous estrogens.

There are great similarity and differences between human and the rat temporomandibular joint from anatomical and histological structure.

The rat temporomandibular joint (TMJ) shows great similarity to the human TMJ, both macroscop-

ically and histologically^(1,2). The human TMJ has an articular eminence in the temporal bone, whereas no articular eminence is found in the rat TMJ⁽²⁾. The rats also have a larger angle between the mandibular corpus and the condyle axis than humans⁽²⁾. Though these species variations in the anatomical features between the rats and humans, however, the rat TMJ still suitable for comparable studies for TMJ.

The sex hormones are cholesterol derived steroid secreted primarily by the male and female gonads and their secretions are tightly controlled by the hypothalamic pituitary axis⁽³⁾.

The testosterone is the principal male sex hor-

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and the anabolic steroid the main sex hormone produced in the males and has several functions including the spermatogenesis, growth, aggression and cardiovascular health. It is found in humans and other vertebrates. In humans and other mammals, the testosterone is secreted primarily by the testicles and, to a lesser extent, by the ovaries. However, small amounts of testosterone are also secreted by the adrenal glands. In the adult males, the levels of testosterone, on average, are about 7 to 8 times as great as in the adult females⁽⁴⁾.

Estrogen is the primary female sex hormone as well as a medication and it is responsible for the development and regulation of the female reproductive system and secondary sex characteristics⁽⁵⁾. Estrogens are used as medications as part of some oral contraceptives, in hormone replacement therapy for postmenopausal, hypogonadal, and transgender women, and in the treatment of certain hormone sensitive cancers like prostate cancer and breast cancer⁽⁷⁾. The three major naturally occurring forms of estrogen in the women are estrone (E1), estradiol (E2), and estriol (E3). Another type of estrogen called estetrol (E4) is produced only during the pregnancy.

The reproductive cycle in female rats, called estrous cycle, is characterized as proestrus, estrus, metestrus or diestrus I and diestrus or diestrus II phases. The ovulation occurs from the beginning of proestrus to the end of estrus. The mean cycle length in the female rat is 4 days, and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle⁽⁷⁾. During the estrous cycle, the prolactin, LH and FSH remain low and increase in the afternoon of the proestrus phase. The estradiol levels begin to increase at metestrus, reaching its peak levels during the proestrus and returning to the baseline at the estrus⁽⁷⁾.

The studies have shown that females make up the

majority of TMDs patients, having more symptoms and clinical signs than men⁽⁸⁻¹²⁾. Since women have higher frequency and severity of TMDs than men, and so they seek for the medical treatment three times more often than men. Therefore, the women are more likely to have persistent TMD symptoms than men⁽¹³⁾. Moreover, the severity of symptoms is also related to the age of the patients where the pain onset tends to occur after puberty, and the peaks in the reproductive years, with the highest prevalence, were occurring in women aged 20–40⁽¹⁴⁾. Whereas the lowest TMDs were among children, adolescents, and the elderly women⁽¹⁵⁾.

In the ovariectomized animals, the thickness of the articular soft tissue was increased by the anterior and central portions of the condyle, whereas the bone volume was decreased in the posterior portion. It was indicated that estrogen deficiency in rats during puberty predisposes to alterations of the TMJ through changes in serum calcitonin and parathyroid hormone levels. Kuroda's study revealed the bone mineral density of the condylar region and the trabeculae of the mandibular bone were decreased in the ovariectomized rats and the decrease of bone density will lead to the degeneration of TMJ⁽¹⁶⁾.

Some studies have suggested a role of the estrogenic hormones in the development of cartilage pathology on osteoarthritis. The estradiol administration has been shown to be more associated with articular the cartilage erosion of greater frequency and severity than in placebo treated controls. This effect of estradiol on joint pathology was accompanied by an effect on cartilage metabolism, with a significant reduction in sulfated proteoglycan synthesis of articular cartilage in the estradiol treated rabbit⁽¹⁷⁾. The studies on articular chondrocytes in cell culture have demonstrated that the estradiol stimulates chondrocyte prostaglandin synthesis⁽¹⁸⁾.

Another possible hormonal factor may be relaxing in, a female polypeptide hormone produced by

the corpus luteum. It is thought to cause muscle relaxation by increasing the expression of specific tissue degrading enzymes. The estrogen may strengthen the effect of relax in and incur the degradative remodeling of the articular disc. The Laxity of the TMJ is thought to play a role in the development of TMDs and suggests a mechanism by which the combined effect of relax in and estrogen may predispose women to TMDs⁽¹⁹⁾.

The synovial membrane in TMJ contain macrophage like type a cells and fibroblast like B cells and the estrogen receptor can be detected in the fibroblast-like B cells which suggesting that the TMJ is a target tissue for estrogen⁽²⁰⁾. The collagen and elastin make up a great deal of the articular disc, and both are often found to be altered in the presence of TMD symptoms. A study of castrated rats indicated that steroid sex hormones do have an effect on the collagen and protein content of the TMJ disc; and it was concluded that the collagen content of the TMJ disc in the normal rat is dependent on the animal's sex⁽²¹⁾.

MATERIALS AND METHODS

This study was carried out on total of 42 male and female Sprague-Dawley strain rats. The weight of male and female rats ranges from 180–250 g at the time of arrival to the animal house with age from 3–4 month. These features suit well for all the experimental animals. The rats had free access to stock water and dry food free from soy bean meal since the soy oil contain estrogen hormone.

The animals were divided randomly into control group (G I): consisted of 14 male and female animals, seven rats each, were subjected to sham operation. Ovariectomy group (G II): consisted of 14 female rats which were subjected to ovariectomy. Testectomy group (G III): consisted of 14 male rats which were subjected to testectomy. the ovariectomy with estrogen replacement group (G

IV): consisted of 7 rats from the ovariectomized female rats which were replaced with estrogen. The testectomy with testosterone replacement group (G V): consisted of 7 rats from the testectomized male rats which were replaced with testosterone.

Surgical interference of gonadectomy: was performed for the male and female rats were anaesthetized with an intramuscular injection of a mixture of ketamine (55 mg/kg) and xylazine (5.5 mg/kg). The ovariectomy was achieved via bilateral flank incisions. The ovarian bundles were ligated with 4-0 silk sutures and removed. The fascia and the skin were closed with 5-0 silk sutures. The orchietomy was performed through a single scrotal incision; the testes were ligated with 4-0 silk suture and removed. The skin was closed with 5-0 silk suture. The sham operation was performed to animals underwent surgical procedures similar to that of the gonadectomized animals, except that the gonads were not removed.

After 50 days from surgery the animals were treated as follow: - (a) Seven ovariectomized animals of group II were injected with estradiol (Folone- misr pharmaceutical company- Egypt) intramuscularly (IM) with dose of 0.5 mg/kg body weight every 7 days for 4 weeks. (b) Seven testectomized animals of group III, were injected with testosterone (Cidotestone-chemical industries development (CID) Giza, A.R.E) IM with dose of 20 mg/kg body weight every 7 days for 4 weeks.

The estrogen or testosterone hormonal levels in the serum were measured in the corresponding animals including: (1) gonadectomized rats, (2) gonadectomized rats supplemented, with the proper hormonal replacement therapy and (3) control rats at the proestrus phase of estrus cycle according to Marcondes.⁷ The hormonal measurement was determined after seven days from the last injection. The data was recorded and statistically analyzed by: (1) one-way analysis of variance (ANOVA) used for

comparison between hormonal level measurements in different groups. (2) Tukey's post hoc test was performed when ANOVA test revealed significant differences between the levels of different hormones.

RESULTS

(1) Histological studies

The control group showed the histological component of the TMJ as condylar fibrous layer of glenoid fibrous layer. Condylar cartilage layer and glenoid cartilage layer. Temporodiscal space and condylodiscal space. Subarticular bone marrow. The fibrous layer, covering the condylar head consisted of numerous fibroblasts scattered through dense parallelly arranged avascular layers of collagen fibers (Fig.1).

The histological changes in TMJ induced by (gonadectomy and hormonal replacement) the TMJ after ovariectomy showed central fusion of articular disc to the fibrous and cartilaginous layers of mandibular condyle (Fig.2) and irregular thickness of condylar and glenoid cartilaginous layers associated

with the condensation of chondrocytes. Close and frequent rapprochement of bone marrow spaces in mandibular condyle close to condylar surface. Widening of condylodiscal space at posterior band. Development of lymphoid follicles in synovial membrane (Fig.3).

The TMJ of testectomized rats showed defasciculation of fibrillar elements of articular disc with its invasion by fat cells. Interlacing of articular disc fibrils with those of condylar fibrous layer. Condylar proliferative layer is well developed but condylar cartilaginous layer is thin and irregular with few and atypical chondrocytes (Fig.4). Fusion of articular disc with fibrous layer of glenoid roof. Numerous bone marrow spaces reaching close to condylar surface. Proliferation of synovial villi with their invasion by numerous vascular sinusoids, fat cells or lipid-filled macrophages to synovial subintima and the posterior band of articular disc.

The TMJ of ovariectomy with estrogen replacement showed dense condylar fibrous layer. Irregular thickness or even absence of condylar cartilaginous layer with development of atypical chondrocytes. Cortification of subarticular condylar bone with de-

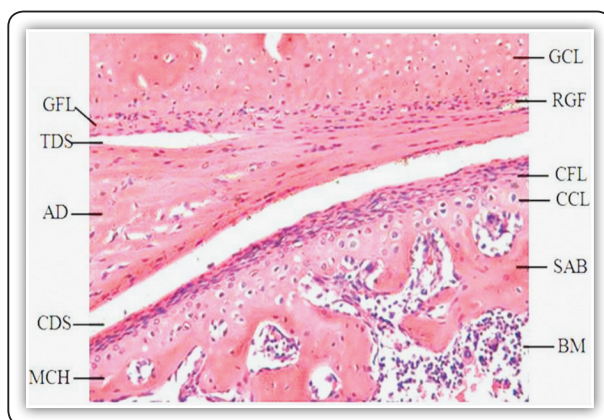


Fig. (1) Male rat TMJ of control group (GI) showing its histological structural components. (H & E stain, orig. mag. X400). CFL: Condylar fibrous layer GFL: Glenoid fibrous layer. CCL: Condylar cartilage layer. BM: Bone marrow. GCL: Glenoid cartilage layer. TDS: Temporodiscal space. CDS: Condylodiscal space. SAB: Subarticular bone.

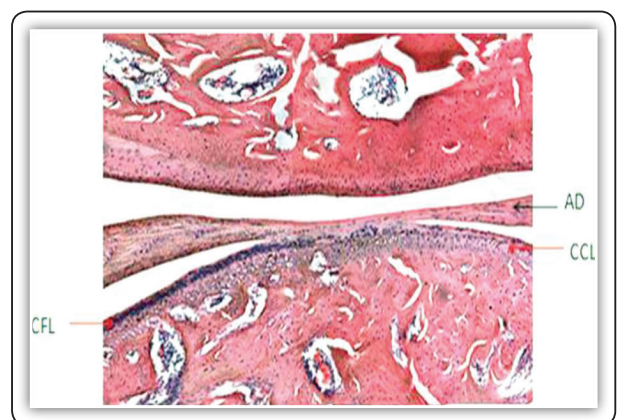


Fig. (2) TMJ of ovariectomized rat (G II) showing fusion of central part of articular disc (AD) to condylar fibrous layer (CFL) with irregular thickness of condylar cartilage layer (CCL) and condensation of its chondrocytes. (H & E stain, orig. mag. X100)

velopment of densely arranged haversian systems and few bone marrow spaces (Fig.5).

The TMJ of testectomy with testosterone replacement showed intermission of condylar cartilaginous layer with the developments of atypical chondrocytes. Central thinning of cartilaginous lay-

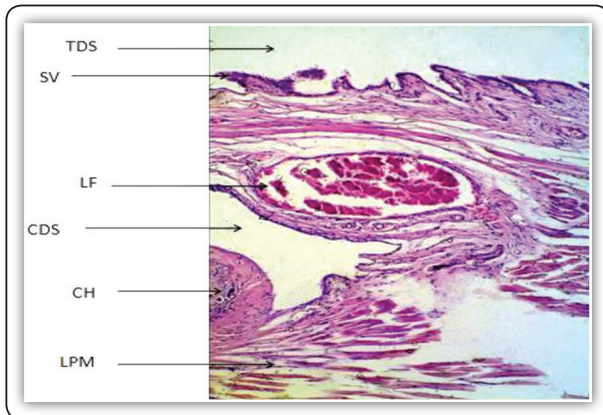


Fig. (3) TMJ of ovariectomized rat (G II) showing the posterior band of articular disc seen at the left part of fig 10, showing lymphoid follicle (LF) containing several discrete aggregates of lymphocytes with several blood vessel below. Some fibers of lateral pterygoid muscle (LPM) are seen attaching condylar head (CH) (H & E stain, orig. mag X100) SV: synovial villi. CDS: condylodiscal space. TDS: Temporodiscal space. LPM: lateral pterygoid muscle. LF: lymphoid follicle CH: Condylar head.

er and fibrous layer of mandibular condyle. Irregular thickness of the fibrous and cartilaginous layers of glenoid roof. Developments of atypical chondrocytes and posterior defasciculation of articular disc. Cortification of subarticular condylar bone reflected by densely formed haversian systems (Fig.6).

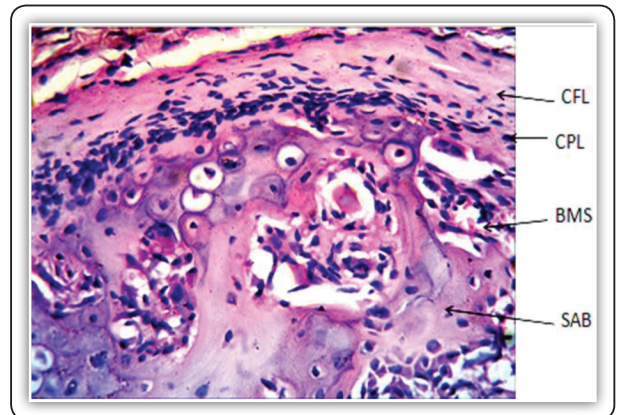


Fig. (4) Mandibular condyle of testectomized rats (GIII) showing irregular thickness of condylar proliferative layer (CPL) and absence of cartilaginous layer except remnants seen as small cartilage islands with atypical, few and irregularly distributed unpaired chondrocytes. (H & E stain, orig. mag X400) CFL: condylar fibrous layer. BMS: bone marrow spaces. SAB: subarticular bone.

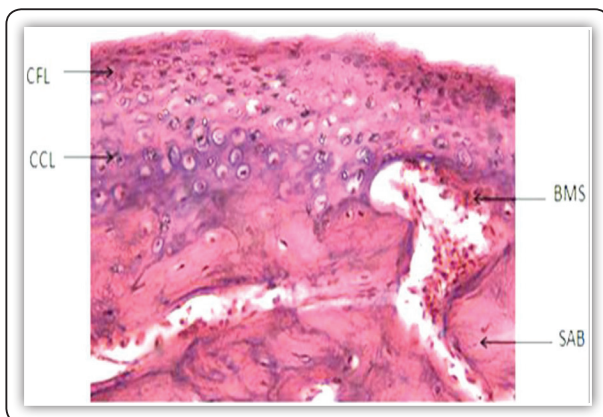


Fig. (5) TMJ of ovariectomized rat with estrogen replacement (G IV) showing interference of the condylar fibrous layer (CFL) with irregular condylar cartilaginous layer (CCL) containing pyknotic chondrocytes. The bone marrow spaces (BMS) invaded the overlying layers of condyle while the condylar subarticular bone (SAB) appears sclerotic with many reversal lines. (H & E stain, rig. mag X400)

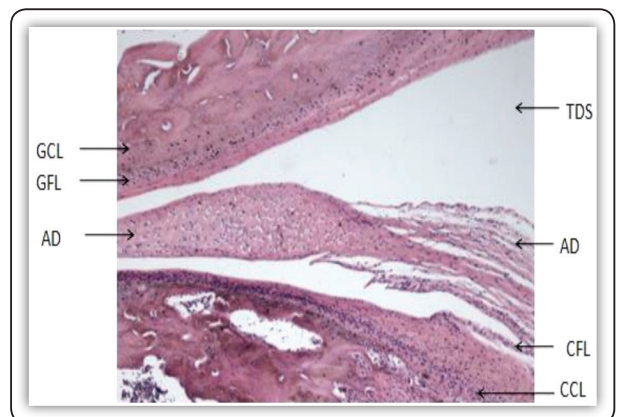


Fig. (6) TMJ of testectomized rat with testosterone replacement (GV) showing posterior fibrillar defasciculation of articular disc (AD) and them interlace with condylar fibrous layer (CFL). Condylar fibrous layer (CFL) and condylar cartilaginous layer (CCL) appear thin centrally (right) but thick posteriorly. The glenoid cartilaginous layer (GCL) and the glenoid fibrous layer (GFL) become thick and highly cellular. (H & E stain, orig. mag X 100).

(2) Statistical analysis:

The statistical analysis of estrogen levels in the diverting animal groups has indicated that the means of estrogen levels were higher in the control animals. However, the means of the estrogen levels after ovariectomy have been extensively reduced. The mean values of hormonal levels after hormonal replacement have been significantly elevated in ovariectomized rat but they did not reach those in the control levels (diagram 1). The same configurations were also observed in respect to the mean values of testosterone in the different groups of animals (diagram 2).

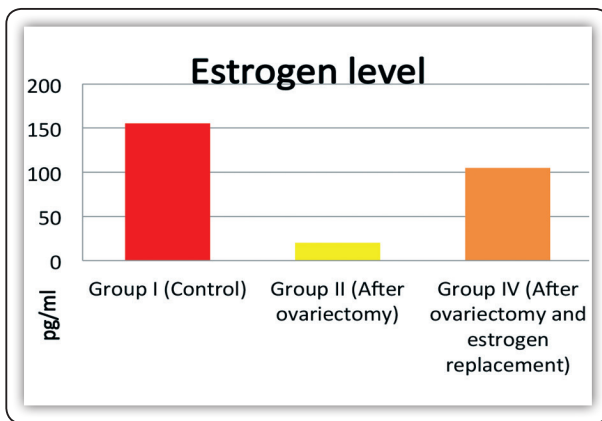


Diagram (1): bar chart representing the means of estrogen levels in the different animal groups

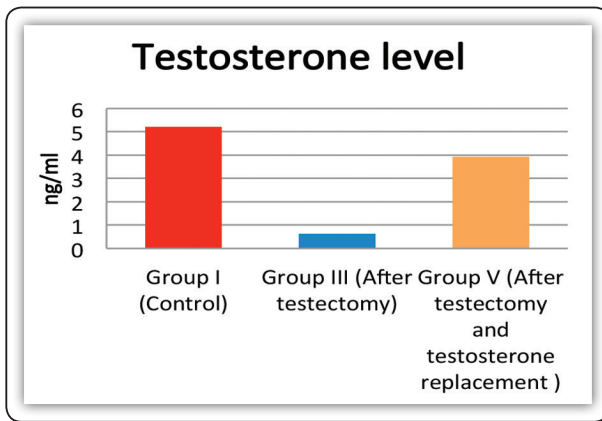


Diagram (2): bar chart representing the means of testosterone levels in the different animal groups.

DISCUSSION

Literally, the temporomandibular disorders (TMDs) are defined as a wide group of pathological conditions involving pain and dysfunction in the masticatory system. It is a multifactorial condition that has different signs and symptoms in the joints and its structural components ⁽²²⁾. Several studies presumed that the inflammatory disorders are considered as the inducing mechanism underlying many of the clinical symptoms referred to as arthritis. Its manifestations include group of variable inflammatory disorders in which the degenerative joints changes simulate the outstanding feature ^(23,24).

The present study has been designed to investigate the effects of estrogen and testosterone deficiency and their subsequent replacement on the TMJ of female and male rats. Though the rats have no articular eminence ⁽²⁵⁾, however, the rats were used as suitable experimental for TMJ studies animals due to close morphological and histological similarity of the TMJ articular structures of the rats to those of the humans.

Effect of decreased level of estrogen on TMJ: the present work has proved that the TMJ of the ovariectomized rats underwent variable microscopical changes in response to the estrogen deficiency. The most interesting finding was the fusion of the central part of the articular disc to the fibrous and cartilaginous layers of the mandibular condyle. Also, an irregular thickness of both the condylar and the glenoid cartilaginous layers were seen associated with the condensation of chondrocytes. However, the chondrocytes of the condylar cartilage were atrophied as evidenced with their variable reduction in size and pyknotic nuclei.

These histological changes were accompanied with reduction in the hormonal measurements that were a statistically significantly different from those measurements in the control rats. Such observations were comparable to the other studies which

explored an intimate relationship between the estrogen deficiency and the histological changes of the TMJ⁽²⁶⁾. These observations also were in the same line with the facts which indicated that the serum estrogen level, decreased by ovariectomy animals, can lead to the increase thickness of the articular soft tissue at the anterior and central portion of the condyle at 1,2,3 weeks after the ovariectomy⁽²⁷⁾.

The previous studies have reported that the estrogen can affect the chondrocytes of cartilage and indicated that this effect may be directly occurred through the estrogen hormone receptors ER α and ER β , and possibly indirectly by modulating the production of cytokines, matrix metalloproteinases and growth factors⁽²⁸⁾. They found that ER α and ER β are expressed in chondrocytes and subchondral bone⁽²⁹⁾. Other studies revealed that the ovariectomy can induce articular cartilage degradation and demonstrated also histopathological features of osteoarthritis in the knee joint cartilage both of the rats and the cynomolgus monkeys⁽³⁰⁾. The ovariectomy was found to increase the thickness of condylar cartilage in young rats⁽²⁷⁾. Additionally, ER beta knockout mice have shown thicker mandibulocondylar cartilage with an increased number of cartilage cells, signifying the direct effect of estrogen on the condylar cartilage⁽³¹⁾.

One other most interesting finding of the present study was the frequent rapprochement of bone marrow spaces in mandibular condyle close to the condylar surface and the widening of the condylodiscal space. These results may be comparable with those noted in another research that found invasion of the trabecular bone into the articular soft tissue in the posterior portion at 2 weeks after surgery⁽³²⁾. They indicated that the subchondral bone involvement may occur at a relatively early stage. They believed that the site-specific differences in the skeletal response to estrogen deficiency has been related to the differences in the metabolic activity, blood supply, osteoprogenitor cell populations, and the

base line levels of bone turnover magnitude experienced by the bone cells⁽³²⁾. Therefore, the effects of ovariectomy may vary in different locations and these changes may accordingly represent an impaired bone turnover after ovariectomy⁽³²⁾.

According to other histological study, observed increase in the thickness of condylar cartilage in ovariectomized rats, was partly due to a change in the number of osteoclast and or their activity in the cartilage-bone interface. The thickness of condylar cartilage accumulatively increases when osteoclasts are fewer in number, resorbing the less mineralized matrix of the hypertrophic layer, making invasion by blood capillaries from underlying bones lower, and finally resulting in slower replacement of cartilage with bone.³³ These are contradictory to our finding which found irregular thickness of the condylar and the glenoid cartilaginous layers seen associated with the condensation of chondrocytes. In the current research, the development of lymphoid follicles in synovial membrane represent further interesting feature denoting the persistence of chronic inflammation and this may simulate the only apparent feature denoting the presence of chronic inflammation. In general, the arthritis is defined as joint inflammation accompanied by increased amount of synovial fluid due to the associated inflammation of synovial membrane (synovitis). Most important types of chronic arthritis are juvenile idiopathic arthritis (JIA), oligoarthritic, rheumatoid factor (RF) positive polyarthritis, rheumatoid factor negative polyarthritis, rheumatoid arthritis and osteoarthritis.

On the other hand, other studies considered the osteoarthritis as non-inflammatory joints disease but rather it is degenerative disease which may simulate accentuation of the normal aging process of the articular cartilage⁽³⁴⁾. The constant wear and tear on the joint after much year activity causes gradual deterioration of the central part of articular cartilage. Alternatively, the metabolic factors may be also implicated as the etiologic factor for joint degenera-

tion. It is disease of later life and it begins by softening and fraying of articular cartilage to become progressively thinner and ultimately exposure of the underlying bone and inexplicable proliferation of cartilage cells at the margin of articular area while the new cartilage formed soon ossifies⁽³⁴⁾. These are comparable with the observed thinning or even absence of the condylar cartilaginous layer with rapprochement of bone trabeculae close to condylar surface noted in the present findings which may indicate either failure of condylar cartilage genesis or precocious endochondral ossification.

Effect of decreased level of testosterone on TMJ: in the current study, the TMJ of testectomized rats has undergone different histological changes in response to the testosterone deficiency when compared with the control group. These features were manifested by the defasciculation of the fibrillar elements of the articular disc with its invasion by fat cells and the interlacing of articular disc fibrils with those of the condylar fibrous layer. These histological results were comparable with the hormonal analysis in which there was a statistically significant reduction in the testosterone level than that recorded in the control animals.

Similar changes to those observed in the ovariectomy group were seen in the present study as the well-developed condylar proliferative layer though the condylar cartilaginous layer was thin and irregular with few and atypical chondrocytes. The fusion of articular disc with the fibrous layer in the roof of the glenoid fossa and the numerous bone marrow spaces reaching close to the condylar surface were noted in the present investigation.

Several studies have suggested that the testosterone has immune modulating properties and may suppress the expression of the proinflammatory cytokines TNF α , IL-1 β , and IL-6 and it can also potentiate the expression of the anti-inflammatory cytokine IL-10.⁽³⁵⁾

In the testectomized rats, the synovial membrane noted in the present study was highly invaded by vascular sinusoids and fat cells or lipid-filled macrophages to the synovial subintima associated with the proliferation of synovial villi. These histological alteration in the TMJ noted after testectomy, may be either due to chronic inflammatory or degenerative or reparative condition.

Several immunological abnormalities have been reported in the juvenile idiopathic arthritis and many of which were similar to those reported in the adult rheumatoid arthritis and the inflammatory synovitis, in juvenile idiopathic arthritis, is similar to that seen in adult rheumatoid arthritis⁽³⁶⁾. The synovium showed pronounced hyperplasia of the lining layer and exuberant infiltration of the subliming layer with the mononuclear cells, including T-cells, β -cells, macrophages, dendritic cells and plasma cells⁽³⁾. The hypertrophied synovial tissue in the present study was noted to be invaded by many blood sinusoids. However, the proangiogenic, vascular endothelial growth factor is believed to be highly expressed in the synovial tissue and osteopenic was noted to be raised in the synovial fluid and the synovial tissue and this was correlated with the new vascularization⁽³⁷⁾.

Effect of estrogen replacement on ovariectomized rats TMJ: the results of this group of rats showed dense condylar fibrous layer, irregular thickness or even absence of the condylar cartilaginous layer with the development of atypical chondrocytes. The cortification of the subarticular condylar bone was associated with development of a densely arranged haversian systems and few bone marrow spaces. However most of the articular soft tissue as well as the subchondral bone seemed to be almost normal. The hormonal measurements indicated that though there was a statistically significant reduction in these measurements after ovariectomy, however, this reduction was decreased after the estrogen replacement therapy. Therefore, the highest

mean value of estrogen level was found in control group (I), followed by that of estrogen replacement after ovariectomy but the lowest mean value of estrogen level was found in ovariectomized animals.

The beneficial effects of estrogen replacement on the TMJ structure were comparable with the findings seen in many previous investigations reported by the administration of 17 β -estradiol to the ovariectomized rats such as the plasma concentration of hormone was physiological and similar to the concentration observed during the rats estrus cycle.³⁸ These may interpret what was happened in the present study when the estrogen level has increased in the ovariectomized rats after replacement therapy with the exogenous hormone though this increase did not reach that level in the control animals.

The previous investigations studied the estradiol effects on the proliferation and metabolism of rabbit mandibular condylar cartilage cells in vitro and they found that the estradiol administration was more associated with the articular cartilage erosion but with greater frequency and severity than that in the placebo treated control⁽¹⁷⁾. These findings are comparable to the present findings who demonstrated the absence of the condylar cartilaginous layer with the development of atypical chondrocytes. The estradiol effect on joint pathology was accompanied by the effect on cartilage metabolism and a significant reduction in sulfated proteoglycan synthesis of articular cartilage in the estradiol treated rabbits has been presumed⁽¹⁷⁾.

Effect of testosterone replacement on testectomized rats TMJ: several investigators had studied testosterone replacement potentiality to counteract the effect of hormonal deficiency after testectomy. In the present study, the testosterone replacement did not completely improve the harmful histological features seen in the testectomized rats. These features were characterized by the intermission or thinning of condylar cartilaginous layer, irregular thickness of both the fibrous and cartilaginous lay-

er, defasciculation of articular disc and the cortification of subarticular condylar bone as reflected by the densely formed haversian systems. These persistent harmful changes may be due to the statistically significant difference in the testosterone level between the control animals and that after testosterone replacement therapy. The highest mean value of testosterone level found in control animals has not been fully restored with testosterone replacement to the testectomized rats.

CONCLUSIONS

The present findings, as well as, the previous investigations have proved that the harmful histological changes on TMJ structure occur with the decreased level of estrogen and testosterone. The therapeutic effect of estrogen and testosterone replacement have not improved completely the harmful histological changes on TMJ after gonadectomy. The effect of decreased level of the testosterone on TMJ was more than the estrogen and there is not considerable improvement of TMJ disturbance was observed when the animals were treated with hormone replacement therapy. The animals that were injected with the exogenous estrogen or testosterone replacement have induced the increase in the level of hormones in blood after deficiency in the gonadectomized animals but it did not reach that level of control animals.

Recommendation

The use of exogenous hormones can be used for the treatment of the harmful effect resulting from hormonal deficiency. Further studies are necessary to determine the mechanism which effect histological structure of TMJ after decrease level of estrogen in female rats and deficiency of testosterone in male rats. Other studies are needed to evaluate the potentiality of exogenous estrogen and testosterone replacement therapy on the harmful effect induced in components of TMJ after gonadectomy.

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