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Research Article

Does Psychological Stress Cause A Comparable Histological Effect on Temporomandibular Joint Versus Knee Joint?

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Abstract

Background: Although studies proved that psychological stress (PS) is perpetuating factor in temporomandibular disorders (TMD), it is clinically unethical to prove the role of PS as initiative factor in TMD. A Previous study showed the ability of PS to induce TMD in rat model. However, the distinctive effect of PS on TMJ versus other joints will augment the specificity of PS to induce TMD. **Aim:** Compare the effect of PS on the TMJ versus knee joint in rats. **Study Design:** A 32 male albino rats was randomly divided into 3 groups, control group (CG), foot shock group (SG) and psychological stress group (PSG). SG was merely used to induce stress for PSG and did not participate in analysis, through the communication box chambers. After 4 weeks, PSG and CG were anesthetized, right joints of TMJ, and knee were prepared for histological study. **Results:** Histological examination of the right knee joints of PSG showed intimal thickening, surface irregularity, thinning of cartilage, degenerated chondrocytes. On other hand, TMJ showed matrix discontinuity, degenerated bone trabeculae, areas of superficial ossification, and reduced cellularity of fibrous layer of mandibular cartilage. Also, there was a highly significant destructive morphometric changes between the PSG and CGs in the TMJ and knee joints in all three examined parameters (mean cartilage thickness, mean area percentage of co1lagen fibers and mean color density of IL-1β immune- expression). **Conclusion:** PS produces histological effects on TMJ and knee joints, however its impact on TMJ is significantly higher.

Key Words: Temporomandibular joint, knee joint, TMD, Psychological stress, Rats.

Introduction

Psychological Stress (PS) can seriously affect various parts of the body.(1) The type and extent of its negative impact varies among organs and systems.(2) Recently, many studies authenticated the relation between PS and TMD. With the advent of RDC/TMD in late 1980‘s, axis II or psychosocial factor has evoked the importance of correlating PS to physical complaints in TMD.(3) Currently most, of TMD patients suffer from anxiety, depression, sleep deprivation and other modern stressful conditions.(4,5) Research considered PS as a perpetuating factor that aggravate TMD symptoms.(6)

However, its ability to induce TMD was studied by Barakat (7) who used a rat model to reveal the direct effect of PS on inducing histological changes and alterations in the ultrastructure of the TMJ condylar cartilage and disc. Nevertheless, it has been claimed that PS mechanism of action is merely systemic through the release of inflammatory mediators that affect various body joints including the TMJ.(8)

Conversely, the effect of PS on the TMJ in comparison with other discrete joints has not yet been investigated.(9) If the hypothesis that PS has a special effect on TMJ over other joints can be proven,(10) it will

further elucidate the importance of PS on initiating and perpetuating TMD symptoms. This in turn will change our strategies in management of TMD.[11] Thus, we aim to compare the effect of PS on the TMJ versus knee joint.

Materials and Methods

The study was designed as prospective randomized controlled experimental study to assess the effect of PS on TMJ and knee joint. A thirty-two male albino specified pathogen-free rats aged 1-2 months, weighed 250-300 gm were obtained from the laboratory animal center, faculty of medicine, Minia University. All rats were acclimated to laboratory conditions one week before the experiment with a regular diet and drinking tap water: temperature 20–24 °C, relative humidity 30–60%, and 12-hour light cycle. All experimental and animal care procedures were approved by the ethical committee, faculty of Dentistry, Minia University, and performed in accordance with standard laboratory operating procedures according to the guidelines of the International Association for the Study of Pain (IASP) in conscious animals (Ethical approval number: 846).

Grouping: Rats were divided into three groups:

1. Control group (CG) (n=8): Not exposed to any physical or psychological stimulation.
2. Foot shock group (SG) (n=16): Only used to elicit PS for 3rd group by subjecting them to electric shock. They were not included in experimental observations. Eight rats were alternatively used daily to minimize anticipation and prevent adaptation to stress.
3. Psychological stress group (PSG) (n=8): Experienced PS by sensing the suffering of FS group during shocks (hair erection and scream) through the communication box chambers.

Rats of PSG were subjected to PS through widely used communication box described by the first author and others. [7] One week prior to the experiment, SG and PSG were individually confined and placed into each compartment of the communication box for 1h daily without any stressors to acclimatize them to the new surroundings. During the stress stimulation period, the electric shock was introduced to SG for 60 min/d at a fixed time (10:00-11:00 AM). PSG in isolated chambers who do not receive foot shock are likely to experience PS by proximity and witnessing SG during their screaming and jumping resulting from the electric shock, via visual, auditory, and gustatory routes through transparent and porous walls of the chambers. All the parameters were set as reported to make animals reach the state of shock but without visible physical injury.

Preparation of samples:

After 4 weeks, PSG and CGs were anesthetized with Ketamine (70mg/kg) and Xylazine (10mg/kg).[12]. The right knee and TMJ were dissected, their muscles and skin removed, and they were left in 10% buffer for two days. The decalcification process lasted four weeks and involved 5.5 g of ethylene diamine tetra acetic acid (EDTA) in 90 ml of distilled water and 10 ml of 37–40% formaldehyde. Until the specimens were fully soft and decalcified, EDTA was added every two days. Along the middle line, the joints were sliced longitudinally in a sagittal plane. Following dehydration, the specimens were cleaned and embedded in paraffin.

After sectioning at a thickness of 5 µm, the samples were stained with Masson's trichrome stain and H&E[13]. Haematoxylin was used as a counterstain after other sections were cut on positively charged slides and stained with avidin-biotin peroxidase to show how inflame-matory cells responded to interleukin-1 beta (IL-1β)[13]. A 1:100 dilution of a polyclonal antibody against IL-1β was acquired from R&D Systems (Minneapolis, Minnesota, USA). Brown reactions were indicative of positive reactions.

Morphometric and histologic analysis

The following parameters were measured in the CG and PSG, using the right TMJ as well as the right knee joint of all rats. Five sections were included from each rat among which 5 fields were randomly chosen at a constant magnification for each parameter: 1- Mean cartilage thickness of

the femur and mandibular joints, using H&E-stained sections. Thickness was determined by drawing a perpendicular line from the articular surface to the underlying subchondral bone and expressed in micrometers.

2- Mean area percentage of co1lagen fibers in the synovium of knee joint and TMJ using Masson's trichrome stain.

3- Mean co1or density of the IL-1β expression in the synovium of knee joint and TMJ using immune-histochemistry.

Every measurement was carried out at high magnification fields of power (×400). Leica microscope DM 2500 connected to a camera (Leica DFC 295) and Leica Q win V3 image analysis software, at Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. The PC was connected to an Olympus XB microscope- Japan.

Statistical analysis:

All values of the morphometric results were expressed as mean±SD. Statistical differences among groups were determined using independent samples T test. P values < 0.05 were considered statistically significant.

Results

I. Histological results:

knee joint:

H&E sections of CG showed joint cavity with menisci, articular surfaces of tibia, and femur (fig.1a). Femoral cartilage revealed smooth regular surface, with normal thickness. Chondrocytes were arranged into four layers: superficial flat chondrocytes, transitional rounded chondrocytes, radial layer of elongated chondrocytes, and a layer of calcified cartilage that separate bone from cartilage. Tide mark separating the calcified layer from the radial layer (fig.1b). The synovium showed intima with macrophage-like cells, and fibroblast-like cells while subintima contained blood vessels, and fibro-adipose connective tissue (fig.2a). On the other hand, H&E sections of PSG showed discontinuity of the matrix of femoral cartilage with destruction and thinning of menisci (fig.1c). Also, there was an apparent decrease in the cartilage thickness, areas of chondrocytes loss, and tidemark was duplicated. (fig.1d). Also, there was surface irregularity, areas of superficial ossification (fig.1e). Moreover, there was ill-defined layers, degenerated chondrocytes with pyknotic nuclei, and sclerosis of subchondral bone (fig1f). The synovial membrane showed intimal thickening, and some inflammatory cells (fig.2b).

TMJ:

H&E sections of CG showed joint cavity with articular surface of mandibular condyle, small part of temporal bone, and articular disc (fig.3a). The mandibular cartilage revealed regular smooth surface with normal thickness. Chondrocytes were organized into four layers: fibrous layer, proliferative layer, fibrocartilaginous layer, and calcified cartilage layer. Mandibular cartilage thickness appeared normal (fig.3b). Synovial membrane showed thin intima formed of type A and type B synovial cells. The subintima contained fibro-adipose connective tissue (fig.4a). On the other hand, H&E-stained sections of PSG showed thinning of the articular disc (fig.3c). Mandibular cartilage thickness revealed an apparent decrease, discontinuity of matrix, and degenerated bone trabeculae (fig.3d). The fibrous layer exhibited a reduced cellularity (figs.3e&f), and degeneration of chondrocytes (fig.3e). The synovium showed intimal thickening, and some inflammatory cells (fig.4b).

In knee joint, Masson's trichrome-stained sections of CG revealed subintima with minimal greenish stained collagen fibers (fig.5a). In PSG, there was abundant greenish collagen fibers in intima and subintima (fig.5b). In TMJ, the subintima of CG showed minimal greenish collagen fibers (fig.6a). In PSG, the subintima revealed congested blood vessel surrounded by abundant greenish collagen fibers (fig.6b).

In the knee joint, immunohistochemical stained sections from CG showed the intima and subintima with mild positive immune reactivity (fig.7a). In PSG, the intima and subintima appeared with strong positive IL-1B immune reactivity (fig.7b). In TMJ, the intima and subintima of CG revealed mild positive immune reaction (fig.8a). In PSG, intima and subintima appeared with strong positive IL-1B immune reaction (fig.8b).

II. Morphometric results

There was a significant decrease (P=0.007) in the mean cartilage thickness of femoral cartilage in PSG as compared to CG. There was highly significant decrease (0.000) in the mean cartilage thickness of mandibular cartilage in PSG as compared to CG (Histogram1). Also, there was a significant increase in the co1lagen fibers mean area percentage (P=0.05) in PSG as compared to CG in the knee synovial membrane. As regard TMJ synovial membrane, there was a significant increase (P=0.004) in PSG as compared to CG (Histogram 2). Moreover, there was significant increase in the mean co1or density of IL-1β expression (0.000) in PSG as compared to CG in synovial membrane of both knee and TMJ (Histogram 3).

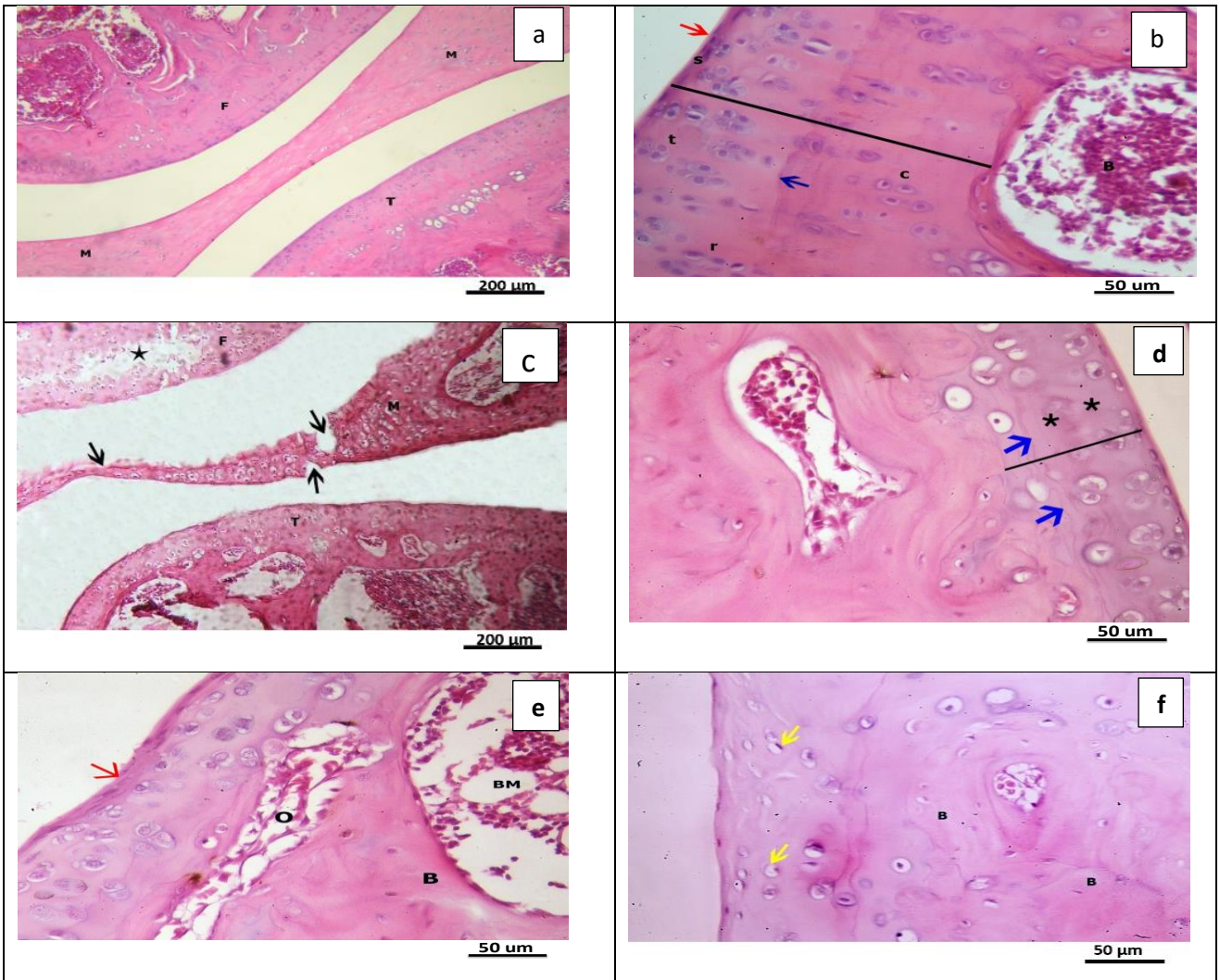


fig.1: photomicrographs of the sagittal section of rat knee joint **(a:CG):** showing joint cavity with menisci (M), articular surfaces of tibia (T), and femur (F). **(b:CG):** showing part of femoral cartilage with a regular smooth surface (red arrow), and normal thickness (black line). Chondrocytes arranged into four layers: superficial flat chondrocytes (s), transitional rounded chondrocytes (t), radial layer of elongated chondrocytes (r), and a layer of calcified cartilage (C) that separate bone (B) from cartilage. Tide mark (blue arrow) separating the calcified layer from the radial layer. **(c:PSG):** showing discontinuity of the matrix (asterisk) of femoral cartilage (F), destruction and thinning of the menisci (M) (black arrow). **(d:PSG):** showing an apparent decrease in the cartilage thickness (black line), areas of chondrocytes loss (*), and tidemark is duplicated (blue arrows). **(e:PSG):** showing surface irregularity (red arrow), area of superficial ossification (O). **(f:PSG):** showing ill-defined layers, degenerated chondrocytes with pyknotic nuclei (yellow arrow),sclerosis of subchondral bone (B). **(H&E X 100 (a,c), (b,d,e,f X400).**

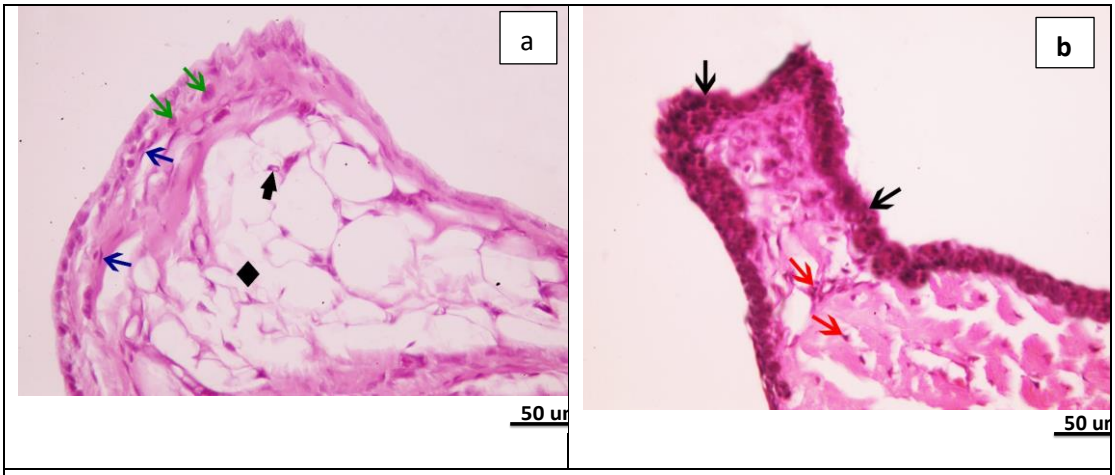
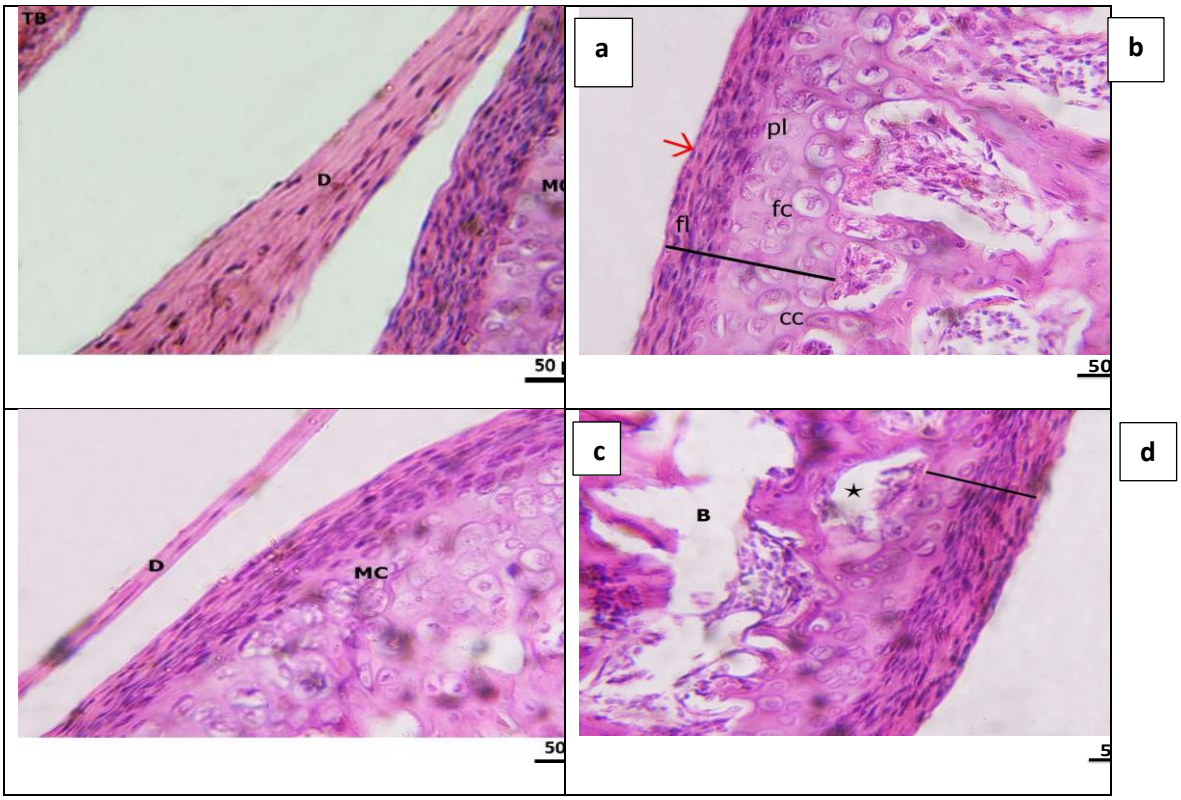


fig.2: photomicrographs of the synovium of rat knee joint. **(a:CG):** showing intima with macrophage-like cells (green arrow) and fibroblast like cells (blue arrow), while the subintima contains blood vesse1s (thick black arrow), and fibro-adipose connective tissue (♦). **(b:PSG):** showing intimal thickening (black arrow) and some inflammatory cells (red arrows). **(H&E X 400).**



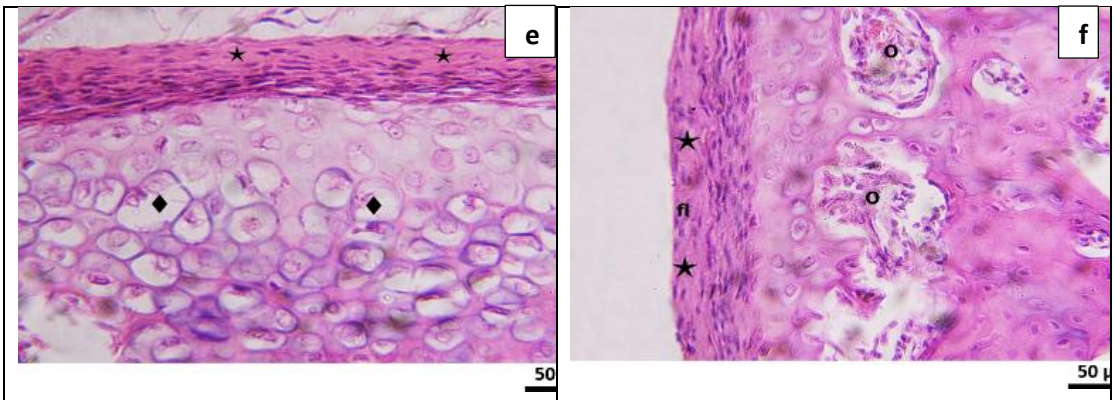


fig.3: photomicrographs of the sagittal section of the rat TMJ **(a: CG):** showing joint cavity with articular surface of mandibular condyle (MC), small part of temporal bone (TB), and articular disc (D). **(b: CG):** showing part of mandibular cartilage with smooth regular surface (red arrow). Chondrocytes are organized into four layers: fibrous layer (fl), proliferative layer (pl), fibrocartilaginous layer (fc), and calcified cartilage layer (cc). Notice, mandibular cartilage thickness appeared normal (black line). **(c: PSG):** showing thinning of the articular disc (D). **(d: PSG):** showing an apparent decrease in mandibular cartilage thickness (black line), discontinuity of matrix (asterisk), and degenerated bone trabeculae (B). **(e: PSG):** showing reduced cellularity of the fibrous layer (asterisk), and degeneration of chondrocytes (♦). **(f:PSG):** showing areas of superficial ossification (o), with reduced cellularity of the fibrous layer (asterisk). **(H&E X400).**

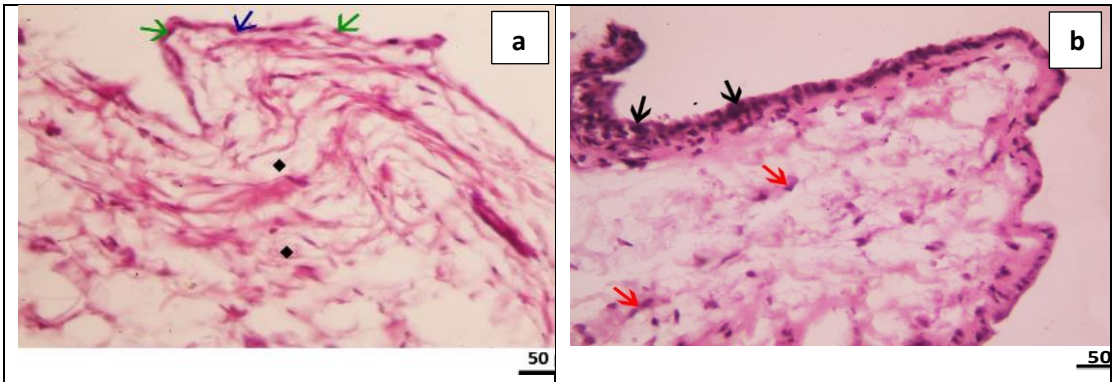


fig.4: photomicrographs of the synovium of rat TMJ **(a:CG):** showing thin intima with type A synovial cells (green arrow) and type B synovial cells (blue arrow). The subintima contains fibro-adipose connective tissue (♦). **(b:PSG):** showing intimal thickening (black arrow), and some inflammatory cells (red arrows). **(H&E X 400).** .

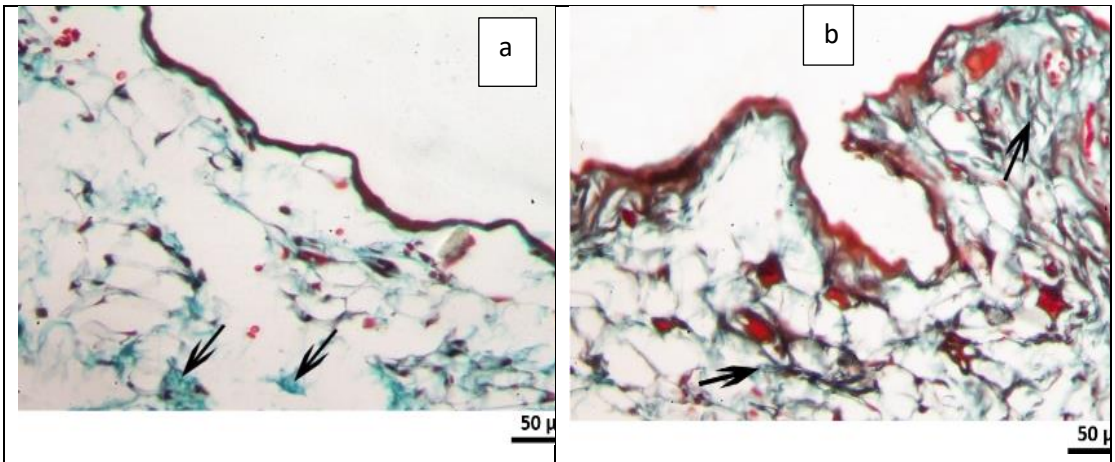


fig.5: photomicrographs of the synovium of rat knee joint **(a:CG):** showing subintima with minimal greenish stained collagen fibers (↑) **(b:PSG):** showing intima and subintima with abundant greenish collagen fibers (↑). **(Masson's trichrome × 400)**

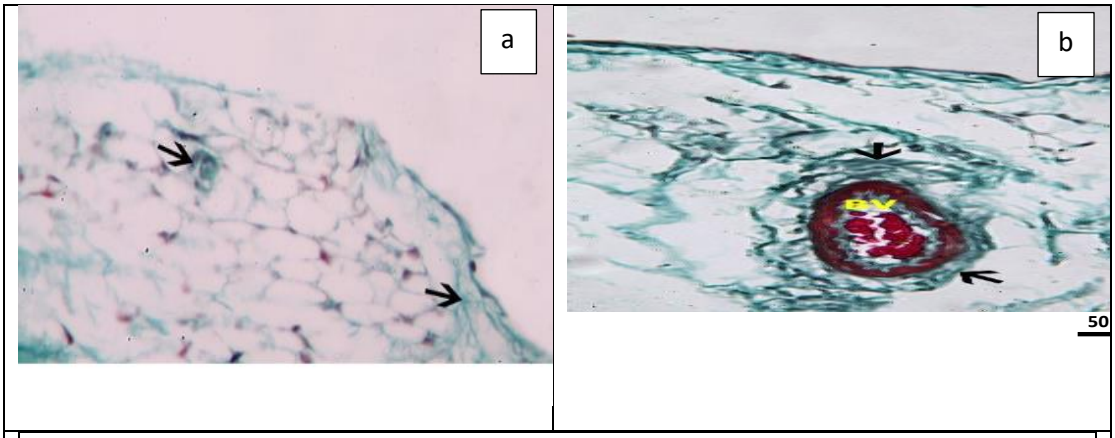


fig.6: photomicrographs of the synovium of rat TMJ **(a:CG):** showing the subintima with minimal greenish collagen fibers (↑). **(b:PSG):** showing subintima with congested blood vessel (BV) surrounded by abundant greenish collagen fibers (↑).
Masson's trichrome× 400)

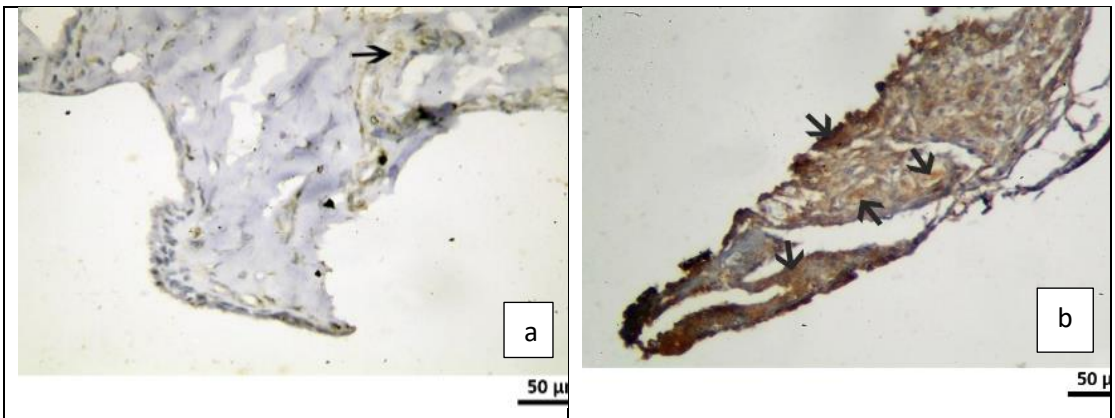


fig.7: photomicrographs of the synovium of rat knee joint **(a:CG):** showing subintima mild positive immune reactivity (↑). **(b:PSG):** showing intima and subintima with strong positive IL-1B immune reactivity (↑). **(Avidin–biotin peroxidase for IL-1B X 400)**

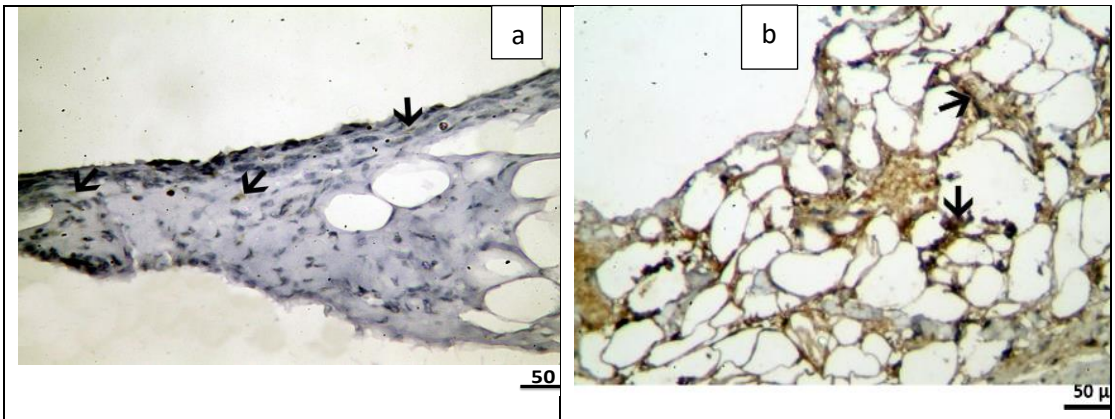
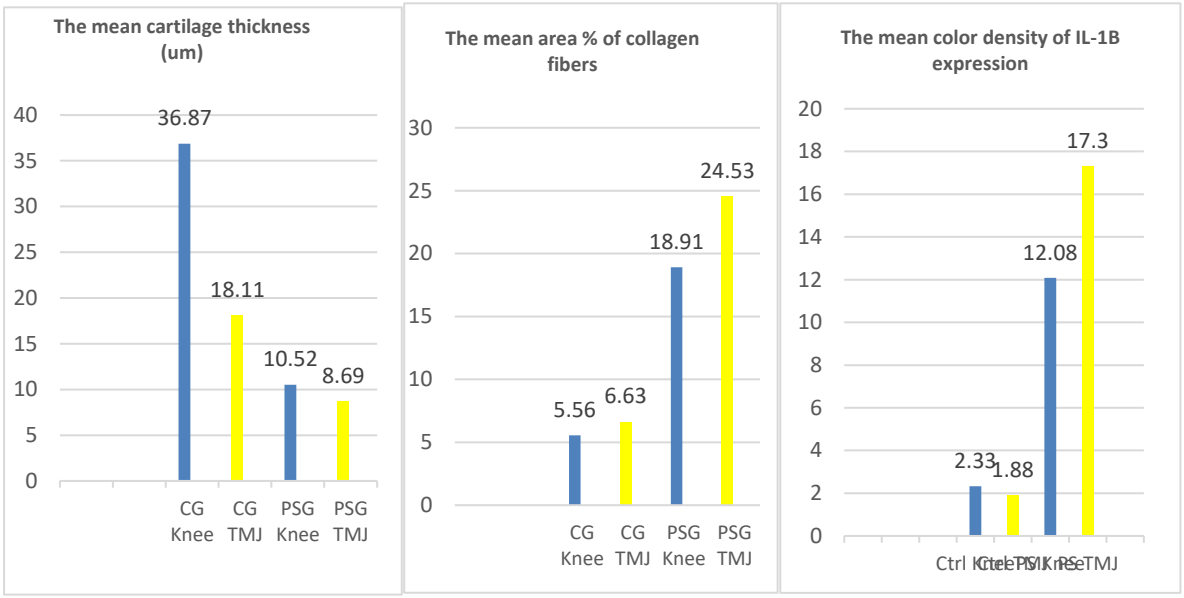


fig.8: photomicrographs of the synovial membrane of the rat TMJ joint of the PS group **(a:CG):** showing the intima and subintima with mild positive immune reaction (↑). **(b:PSG):** showing intima and subintima with strong positive IL-1B immune reaction (↑).

(Avidin–biotin peroxidase for IL-1B X 400)

Histograms



Discussion

Many studies are currently elucidating the effect of psychological factors in the pathogenesis of TMD.[14,15] Although psychological stress is considered as a perpetuating factor in TMD, the ability of PS to induce TMD has not yet been fully proved.[16] The issue has been tested in previous article but sound evidence are still lacking.[7] In the current study we set a hypothesis that PS has a special effect on TMJ over other joints, proving this theory will complement the distinct relation between the PS and TMJ. We implemented rat model and communication box to induce PS, for 1hour daily for four weeks which was previously documented as an effective method and duration for simulating stress and inducing histological changes in TMJ.[17] We noticed behavioral changes of PSG throughout the experiment. PS rats were trying to avoid visual contact with adjacent SG rats, and they significantly reduced their movements and did not

cause abnormal strains on their leg muscles and knee joints.[18] The knee joint was used in comparison with the TMJ as they are considered distant, in addition knee joint is easier in harvesting .[19]

Histological results revealed extensive destruction and histological changes of the TMJ and the knee joints of PSG confirming the damaging effect of PS on the rat joints. These findings were comparable to other studies that established the relationship between PS and inflammatory arthritis [20]. A Recent study showed an increase in the incidence of anxiety disorder before the diagnosis of arthritis, and attributed the destructive articular changes to the stress-induced pro-inflammatory effect, suggesting that inflammation might be the underlying mechanism between the development of inflammatory arthritis and stress.[21-24]
H&E-stained sections of the TMJ of PSG showed thinning of the articular disc, decrease cellularity of the fibrous layer, degeneration of chondrocytes, and an apparent decrease in mandibular cartilage thickness. Also, the subchondral bone showed areas of bone resorption. Other studies related the destructive changes to the spasm of masticatory muscle that occurs due to bruxism observed in anxious rats. However, in the current studies no bruxism was noticed on PSG, they instead showed a calm tendency with infrequent bruxism.[25,26]

The morphometric measurements proved a significant decrease in the thickness of both mandibular and femoral cartilages in PSG which was attributed to degeneration of cartilage which exceeds cartilage synthesis, thus affecting the normal balance [27].

In normal hyaline cartilage growth, the tidemark continues to advance into the noncalcified cartilage with a rate that is similar to the absorption rate of the calcified cartilage from the opposite subarticular bone. Thus, preserving the same thickness of the calcified cartilage throughout life [28]. Interrupted tidemarks in PSG clearly indicated an impairment in the balanced growth rate of the cartilage, and replacement of calcified cartilage by bone [28].

In our present study, PSG showed intimal thickening, infiltration of the subintima with congested blood vessel and some inflammatory cells in both knee and temporomandibular joints. These synovitis results were also shown in another study [29] that showed synovial cell hyperplasia and inflammatory cellular infiltrate in a rat mode1 of OA.

In Masson's stain, H&E examination of PSG revealed an apparent increase in collagen fibers in in the subintima of the synovial membrane of the knee joint and congested blood vessel surrounded by abundant greenish collagen fibers in the subintima of the synovial membrane of TMJ. There was a significant increase in collagen fibers mean area percentages proved by morphometric measurements and statistical analysis. Some authors [30] stated that synovial fibroblast cells were activated to generate large amounts of collagen fibers, resulting in excessive collagen fibers. Moreover, a previous study [31] reported that synovitis might lead to an increase in production of nitric oxide mainly from the endothelium and some inflammatory cells. Hypoxia induced by nitric oxide might enhance permeability to macromolecules, promoting the production of oedema and cell extravasation. This could help to explain osteoarthritis's inflammatory cellular infiltration. Hypoxia might cause synovial angiogenesis, which leads to the formation of new blood vessels and pro-inflammatory cytokines in the synovium.[32]

LM examination of the synovia1 membrane of both knee and TMJ of PSG showed increased expression of IL1-β in the synovial membrane. This result was in accordance with previous study [33] who added that synovial membrane contributes to the degeneration of the joint by releasing inflammatory cytokines. Meanwhile, inflammatory cells might produce reactive oxygen species causing impairment of cartilage structure [34]**.** Cytokines could be involved in promoting intimal thickening of synovium, and inflammation. [35]**.**

Also, IL-1β could activate chondrocytes, and osteoclasts to release metalloproteinases[36] which explain matrix discontinuity, destruction of cartilage, and the underlying subchondral bone.

Conclusion:

Psychological stress alone can cause a destructive effect on joints. However, this damaging potential is significantly higher on TMJ which prove its ability to initiate TMD.

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