Egyptian Journal of Medical Microbiology Volume 28 / No.3 / July 2019 119-129 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Evaluation of Mesenchymal Stem cells as an Adjuvant Therapy in Treatment of Induced Experimental Autoimmune Prostatitis in Albino Rats

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Key words:
Experimental Autoimmune Prostatitis (EAP), Mesenchymal Stem Cells (MSCs), Tacrolimus, Albino rats

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ABSTRACT

Background*: Bone-marrow mesenchymal stem-cells (BMMSCs) efficaciously used in treatment of many immune diseases.* ***Objectives****: The aim of the current study was to investigate the therapeutic value of MSCs in treatment of EAP treated with Tacrolimus.* ***Methodology:*** *25 albino-rats were divided into 5 equal groups (5 rats in each group): Group-I received normal phosphate-buffered saline (Control), Group-II: EAP model with no treatment. Group-III: EAP treated with tacrolimus, Group-IV: EAP treated with systemic MSCs and Group-V: EAP treated with combined tacrolimus and MSCs. The prostatic tissue regeneration investigated through detection of levels of TNF-α and IL-1β gene-expression, histological, fluorescent and immune-histochemical studies.* ***Results:*** *Combined treatment proved to decrease expression of both TNF-α and IL-1β in group-V when compared with the other EAP groups either received treatment or not (groups-II, III, and IV). Photomicrographic examination of the prostatic tissue showed a more evident regeneration in EAP groups (group-IV and V) when compared to tacrolimus group-III. Group-V showed evidence of complete regeneration of most of prostatic acini. The degree of cell apoptosis was evident by strong caspace-3 reaction in EAP untreated group, while the therapeutic effect of MSCs proved by very weak caspace-3 reaction in group-IV and V. The degree of proliferation in the acinar nuclei was evident by strong positive cell nuclear antigen (PCNA) reaction in groups-III, IV, V. No significant difference detected by histomorphometric measurement of both caspase-3 and PCNA among groups-III, IV and V.* ***Conclusion:*** *Our study demonstrated an adjuvant therapeutic effect of MSCs to tacrolimus in treatment of EAP.*

INTRODUCTION

Experimental autoimmune prostatitis (EAP) defined as an induced autoimmune, non-infectious, disease in model rat's male sex accessory glands by immunization with prostate antigen (PAG). EAP is characterized by a prostate-specific, cellular and humoral, autoimmune inflammatory response [1].

Chronic prostatitis (CP) is a common disease of human urogenital system[2]. Chronic pelvic pain syndrome (CPPS) identified clinically by chronic pain in the perineum, rectum, prostate, penis, testicles, and abdomen, which is not concomitant with urogenital infection[3]. CP/CPPS is a frequent health problem, which necessitate proper therapeutic lines. The pathogenesis of CP/CPPS remains indeterminate; however, many studies suggest an underlying autoimmune mechanism, through detection of prostate specific T cell, and inflammatory cytokines in prostatic fluid with no evidence of bacterial infection [4].

EAP has many pathological and immunological correspondences with CP/CPPS in human. Therefore, studies on EAP could aid in understanding the pathogenesis and suggesting possible therapy for CP/CPPS in human [2, 5].

TNF-α and IL-1β are inflammatory mediators, which may display a role in diagnosis and evaluation of possible therapy for EAP [6].

Tacrolimus is one of the successfully used immunosuppressive drugs in treatment of autoimmune diseases and prevention of transplant rejection. Tacrolimus found to be efficiently lessened the inflammatory response in rats with induced EAP [7].

Bone marrow mesenchymal stem cells (BMMSCs) pronounced by its ability to induce tissue repair, rapid cell proliferation, through its characteristic low

immunogenicity, pluripotency, and immunosuppressive effect [8]

BMMSCs efficiently used in treatment of many immune disorders such as graft versus-host disease and Crohn's disease. Injection of BMMSCs significantly modulate the inflammatory reaction, fibrous tissue hyperplasia, and lessen TNF-α and IL-1β expression in model rats with induced chronic bacterial prostatitis (CBP) [9].

Treatment trials with biomarkers could clarify the pathogenesis of chronic prostatic disease and may suggest effective therapeutic regimens. Therefore, the current study performed to investigate if MSCs can supplement the therapeutic efficacy of tacrolimus in treatment of EAP.

METHODOLOGY

- Isolation, culture and labeling of MSCs:

MSCs were attained from the Biochemistry and Molecular Biology Unit, Faculty of Medicine, Cairo University. Bone marrow stromal cells were aspirated from femurs and tibiae of albino rats. The stromal cells were attained by flushing the bone marrow cavity with Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, USA, D5796) supplemented with 10% fetal bovine serum (FBS) (Sigma, USA, F6178). The cells spread over Ficoll-Hypaque (Sigma, USA, F8016) in a ratio of 2:1 in a sterile conical tube and centrifuged. The opaque layer, containing the mononuclear cells, was aspirated and inoculated into culture medium supplemented with 1% penicillin-streptomycin (Sigma, USA, P4333) then incubated at 37°C in 5% humidified CO_2 for 14 days and the media were changed every 3-4 days. When confluent colonies produced (80-90%), cultures were washed twice with phosphate buffer saline (PBS) (Sigma, USA, P5493) and cells were treated with 0.25% trypsin (Sigma, USA, T1426) in 1ml EDTA for 5 minutes at 37°C following centrifugation (at 2400 rpm for 20 minutes), cell pellets were then re-suspended in serum supplemented medium and incubated in 25cm^2 culture flasks. The resultant cultures considered as the first passage cultures [10]

-Labeling of MSCs with PKH26 dye and cell viability analysis:

Culture cells were labeled with fluorescent cell tracker PKH26 (Sigma, USA, MINI26) according to manufacturer's instructions. Cell viability detected by adding 1:1 ratio of cell suspension and 0.4 % trypan blue stain, and examined by phase contrast microscope. Viable cells viewed shiny without staining [11]

-Animals included in this study:

Twenty-five adult male ***Sprague Dawley*** **(SD)** albino rats with an average weight of 200±50 gram were used in this study. They were locally bred at room temperature (18-22˚C) in the animal house at Faculty of Medicine, Cairo University, Egypt according to the international guidelines for laboratory animal care . The experiment protocol, including the animal's treatment, anesthesia and use in experimental research, was approved by the Ethics Committee, Faculty of Medicine, Cairo University. All efforts taken to ensure minimal animal sufferings.

-Induction of autoimmune prostatitis and evaluation of the different therapeutic lines

• *Group I (control group)*

Five SD rats received 1 ml normal phosphate buffered saline (PBS) through subcutaneous injection at multiple points (under the skin of pelvis area of the lower abdomen and the bilateral shoulders) on days 0, 7, 14 and 28.

Induction of EAP model

Twenty SD rats were immunized by subcutaneous injection of 1 ml mixed emulsion PAG and complete Freund's adjuvant (F5881; Sigma-Aldrich; Merck KGaA, Darmsta dt, Germany; 1:1) at multiple points (pelvic area at the lower abdomen and the bilateral shoulders) on days 0, 7, 14 and 28. Then the 20 immunized SD rats divided into four equal groups (group II, III, IV, and V).

- ***Group II*** this group included five PAG immunized rats (non-treated group)
- ***Group III*** (tacrolimus treated group)**.** Five PAG immunized rats, each received Intra-gastric 1 ml tacrolimus (Astellas Pharma Inc., Tokyo, Japan; 0.8 mg/kg), once a day from day 1 in the experiment for thirty consecutive days.
- ***Group IV*** (MSCs treated group)**.** Five PAG immunized rats, each received one systemic injection of MSCs (1 x 10^6) diluted in 0.5 ml of PBS (through the caudal vein) at the day one of immunization (induction of EAP).
- ***Group V*** (combined tacrolimus and MSCs treated group). Five PAG immunized rats each received 1 ml tacrolimus (0.8 mg/kg), by intra-gastric administration once a day for thirty consecutive days combined with one systemic injection of MSCs (1 x 10^6) diluted in 0.5 ml of PBS at the day one of immunization.

The animals were bred individually to evade infection. At the end of the experiment, the animals sacrificed and the prostatic tissue dissected for the following examination:

-TNF-α and IL-1β genes expression were investigated by quantitative real-time polymerase reaction (RT-PCR)

The prostatic tissue conserved at -80°C in PBS (PH 7.4). RNA extraction from tissue homogenate was done by using RNeasy purification kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Complementary DNA produced from 5µg of extracted RNA by using 1 µl (20 pmol) antisense primer and 0.8

µl superscript reverse transcriptase for 1 hour at 37 °C. Relative amounts of mRNA species was studied .PCR primers designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from the Gen-Bank.

The primers used for TNF-α were:

- forward: 5′ -CCCCGACTACGTGCTCCTC-3′ and
- reverse: 5′ -GAACGGATGAACACGCCAGTC-3′

The primers used for IL-1β were:

- forward: 5′ -CAAGGA GAGACAAGCAACGACAA-3′ and
- reverse: 5′ -GTCCCGACCATTGCTGTTTC-3′

Quantitative RT-PCR was conducted in a 25-µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900nM from each primer and 2–3µl of cDNA. Amplification conditions were as follow; 2 min at 50°, 10 min at 95° and 40 cycles of denaturation for 15 seconds and annealing/extension at 60° for 10 min. Comparative threshold cycle method used to investigate the relative expression of the studied genes. The relative quantification was calculated by the expression $2^{-\Delta\Delta Ct}$ [12]

Light Microscopic study:

The specimen was fixed in 10% formal saline solution to prepare paraffin blocks, and cut at 5-6 µm thickness sections. Prostatic tissue sections were deparaffinized in xylol solution and then rehydrated by consecutive use of 100%, 95%, 70% ethyl alcohol. Finally the sections washed in distilled water prior to applying the following stains:

- ***Hematoxylin and Eosin (H &E) staining*.** Was done according to the standard technique then sections were put on cover slips after mounting in Canada balsam [13]
- Immunohistochemistry :
  Prostatic tissue sections (5-6 µm thickness) were deparaffinized by double immersion in xylene, 10 minutes each. Then ethyl alcohol in water used for hydration of the sections. Finally, sections were washed in pure water. Hydrogen peroxide in absolute methanol (0.9% for 10 min.) solution was applied to suppress any endogenous, non-specific, peroxidase activity. Sections were then heated in 10 mM sodium citrate buffer for 30 minutes (in a water bath at 95–100°C), for antigen recovery. Sections washed twice in PBS Tween 20 for 2 minutes, then 5% normal mice serum (at room temperature, for 30 minutes). Sections were incubated with the following primary antibodies for 30 minutes:
  1. *Caspase-3 antibody*, primary antisera diluted (1:500) (Labvision, catalogue number TA-125-UD, Goteborg, Sweden). The peroxidase activity was studied by AEC (3-amino-9-ethyl carbazole) (Labvision, catalogue number TA- 004HAC, Goteborg, Sweden). Apoptotic cells showed brown discoloration of their cytoplasm, as an indication for positive Caspase reaction. Whereas absence of the brown discoloration in the normal cells indicated a negative Caspase reaction.
  2. *Proliferating cell nuclear antigen (PCNA)*, a cofactor of DNA polymerase, was verified by rabbit polyclonal IgG (FL-261; catalogue number SC-7907, 200 µg/ml, dilution 1:50, Santa Cruz Biotechnology, USA). Cell proliferation was demonstrated by brown discoloration of the nuclei; an indication of positive PCNA test. Negative reaction was demonstrated by absence of the brown nuclei [14].
- *Electron Microscopic Examination:*
  Specimens from the prostatic tissue were cut into small slices and prepared for E/M examination as follows. Fixation of specimens was done by applying 4% glutaraldehyde, followed by rinsing in phosphate buffer. Dehydration was followed by consecutive immersion in ethyl alcohol (30%, 50%, 70%, 90%) for five minutes each. Absolute ethyl alcohol was applied twice (ten minutes each) for dehydration of the section. The specimens then immersed in a mixture of equal volume of ethanol (100%) and acetone (100%) for 15 minutes, then in 100% acetone for another 15 minutes twice. For augmentation of tissue infiltration with epoxy resin; specimens were dipped in equal volumes of epon and acetone for one hour. Ultrathin sections (50-60 nm) were obtained by cutting the plastic-embedded tissue into ultra-microtomes (by diamond knives), double staining technique by uranyl acetate, and lead citrate solution was used for staining of the grids. These sections were examined and photographed by a Joel, 100 CX II transmission electron microscope (Jeol, Tokyo, Japan). At first low magnification was used (x1000) to detect the specimen, then, higher magnification used to investigate the qualitative criterion and the morphological changes in the cellular organelles. Images were captured by CCD camera model AMT [15].
- *Histomorphometric measurements:*
  The optical density of the positive Caspase and PCNA reactions was studied by Leica LAS, V3.8 image analyzer computer system (Switzerland). Ten non-overlapping fields per specimen per each of the studied animals were examined, by an independent participant, at a magnification of 400. Image analyzer was designed to convert the optical density measurement units (pixels) into micrometer units [16].

Statistical Analysis

The data obtained for all groups was expressed as mean and standard deviation (± SD) and subjected to statistical analysis using "SPSS 22" software. The data then subjected to analysis of variance using independent t-test. Results considered significant when p -value was ≤0.05.

RESULTS

QRT-PCR for TNF-α and IL-1β:

Expression of both TNF-α and IL-1β was significantly increased in group II, III and IV when compared with group I (control group) and group V which is demonstrating the significance of therapeutic effect of combined MSCs and tacrolimus. However, expression of both TNF-α and IL1β did not show significant variation between group II, III, IV (table1).

Table 1: QRT- PCR products of TNF-α and IL1β in prostate tissue (mean ± SD).

	Group I	Group II	Group III	Group IV	Group V
TNF-α	17.56 ± 2.43	64.34 ± 15.63*	68.36 ± 7.85*	71.53 ± 5.62*	21.74 ± 7.35*
IL-1β	65.41 ± 8.36	348.21 ± 42.37*	338.52 ± 37.25*	314.63 ± 36.26*	69.43 ± 13.56*

*P < 0.05

PKH26 fluorescence stain

MSCs systemically treated groups (group IV and V), one week after treatment, showed PKH labeled red fluorescent cells within the prostatic tissue (Fig 1).

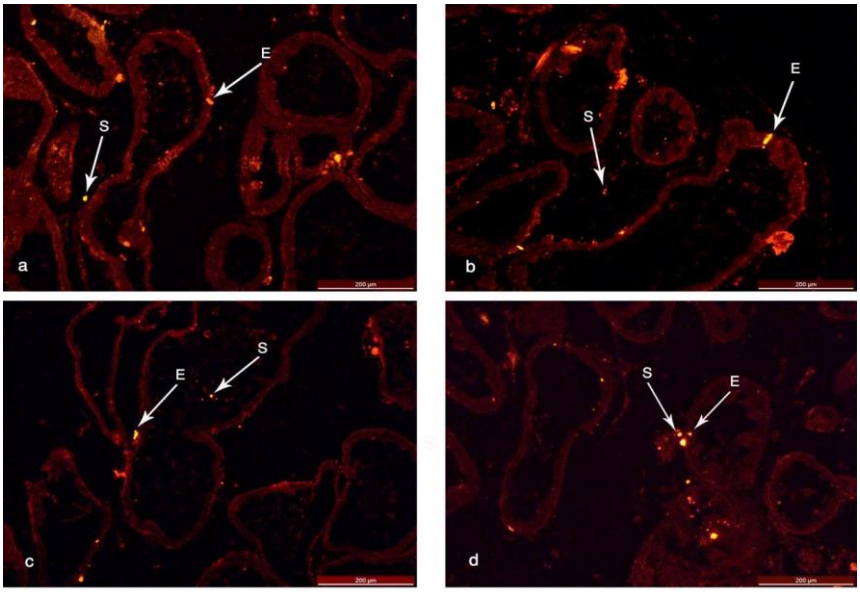

Fig. 1: Photomicrographs of the prostate: a) and b) one week after MSCs treatment (group IV), c) and d) one week after combined MSCs and tacrolimus treatment (group V), showing red fluorescent cells in the proliferated epithelium (E) and in the stroma (S). (PKH, x100).

Light Microscopic Examination

Hematoxylin and eosin stained prostatic tissue examined by light microscopy. Prostatic gland of control group (group I), presented the normal architecture of the glandular tissue. (Fig 2a, b, c). The prostatic glands of group II (untreated group) showed areas of epithelial proliferation without an acinar pattern, the fibromuscular stroma shows fragmentation of the muscle fibers, transudation, dense mononuclear infiltration and interstitial hemorrhage (Fig. 2d). The prostatic tissue of group III showed areas of atrophy in the epithelial lining of the prostatic acini with hyperplasia, the fibromuscular stroma is markedly reduced and is replaced by fatty tissue; the vas deferens is completely normal (Fig. 3a). group IV showed large number of prostatic acini within normal appearance and healthy epithelial lining, few acini contain necrotic debris, the fibromuscular stroma is reduced, with heavy lymphocytic infiltration (Fig. 3b), higher magnification of the last image showing healthy cells lining a prostatic duct with vesicular nuclei, while few cells are vacuolated with pyknotic nuclei and heavy lymphocytic infiltration in the fibromuscular stroma (Fig.3c). Group V showed complete regeneration of most of the acini, with healthy epithelial lining, there are few corpora amylacea, normal fibromuscular stroma, with few lymphocytic infiltration and retracting clump of exudate (Fig 3d).

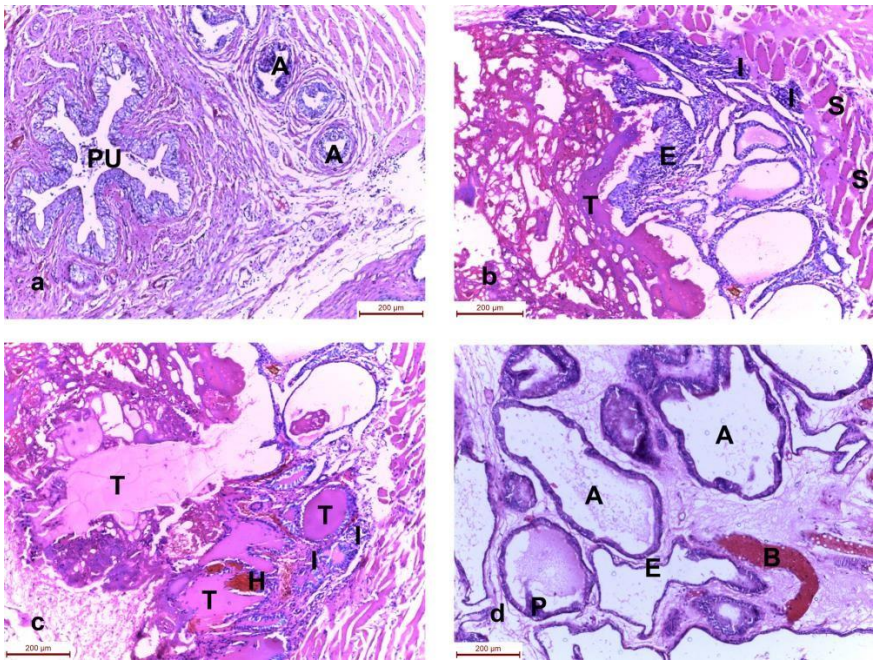

Fig. (2): Photomicrographs of the prostate: (group I) showing normal prostatic acini (A). Normal Prostatic urethra (PU) . b) & c) (group II) showing areas of epithelial proliferation without an acinar pattern (E). The fibromuscular stroma shows fragmentation of the muscle fibers (S), transudation (T), dense mononuclear infiltration (I) and interstitial hemorrhage (H) d) (group III) showing areas of epithelial proliferation in the walls of the acini (A) with formation of papillae (P), and areas of epithelial atrophy (E). The fibromuscular stroma shows marked reduction of the muscular element, with dilated congested blood vessels (B). (H & E x 20).

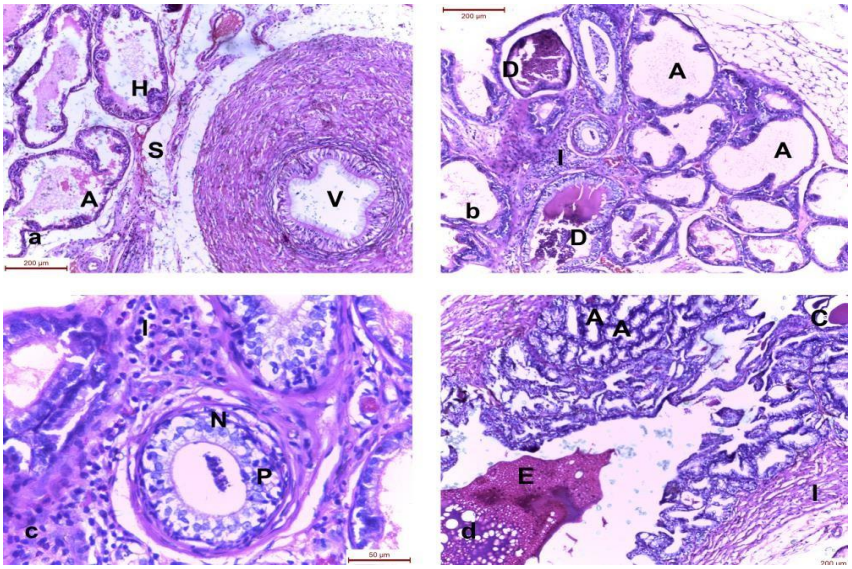

Fig. (3): Photomicrographs of the prostate: a) (group III) showing areas of atrophy (A) in the epithelial lining of the prostatic acini (H). The fibromuscular stroma is markedly reduced and is replaced by fatty tissue (S). The vas deferens (V) is completely normal. b) (group IV) showing large number of prostatic acini with normal appearance (A). Few acini contain necrotic debris (D). The fibromuscular stroma is reduced, with heavy lymphocytic infiltration (I). c) higher magnification of the last image showing healthy cells lining a prostatic duct with vesicular nuclei (N), while few cells are vacuolated with pyknotic nuclei (P) and heavy lymphocytic infiltration in the fibromuscular stroma (I). d) (group V) showing normal appearance of most of the acini. (A). There are few corpora amylacea (C). Normal fibromuscular stroma (I). There is a retracting clump of exudate (E). (H & E a, b & d x 200, c) x 400).

Immuno-historeactivity of Caspase-3 and PCNA

Cellular apoptosis was investigated by immune-histochemical staining with Caspase-3. The specimen of the control group showed very weak caspase reaction in all prostatic acini (Fig 4 a), while group II showed very strong caspase reaction affecting most of the acinar cells (Fig 4 b). Group III showed moderate caspase reaction affecting most of the acinar cells (Fig 4 c). Group IV, V showed very weak caspase reaction (Fig. d, e respectively).

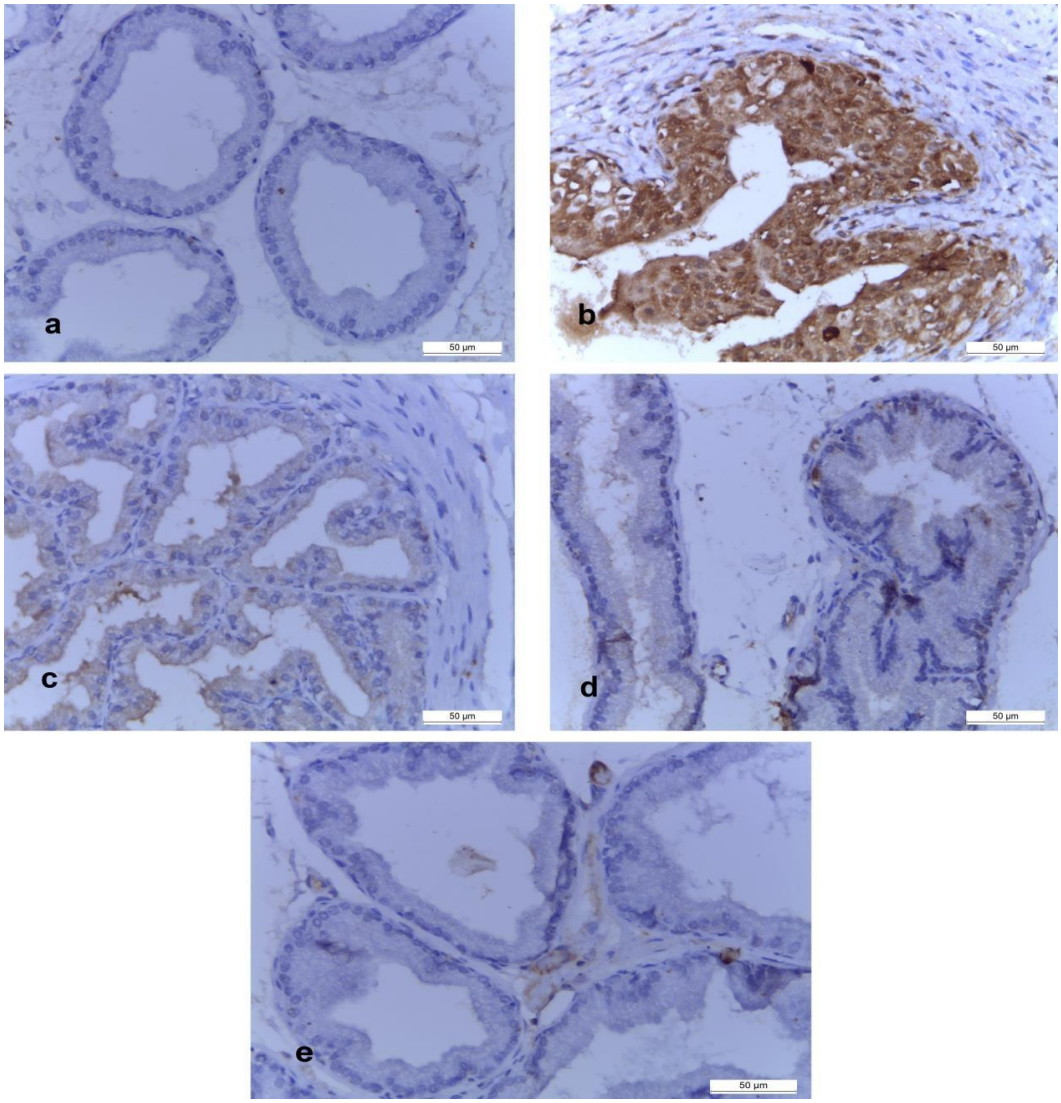

Fig. (4): Photomicrographs of the prostate: a) (group I) showing negative caspase reaction . b) (group II) showing very strong caspase reaction affecting most of the acinar cells (brown color). c) (group III) showing moderate caspase reaction . d) (group IV), e) (group V) showing very weak caspase reaction. (Caspase 3 x 400).

Cellular proliferation of acini was demonstrated by PCNA staining. Group I showed positive PCNA reaction affecting sporadic nuclei within the prostatic acinar cells (Fig 5a). Group II showed weak positive PCNA reaction in some cellular nuclei. While a stronger positive PCNA reaction in most of the cells showed in group III, IV, and V (Fig.5 c, d, e respectively).

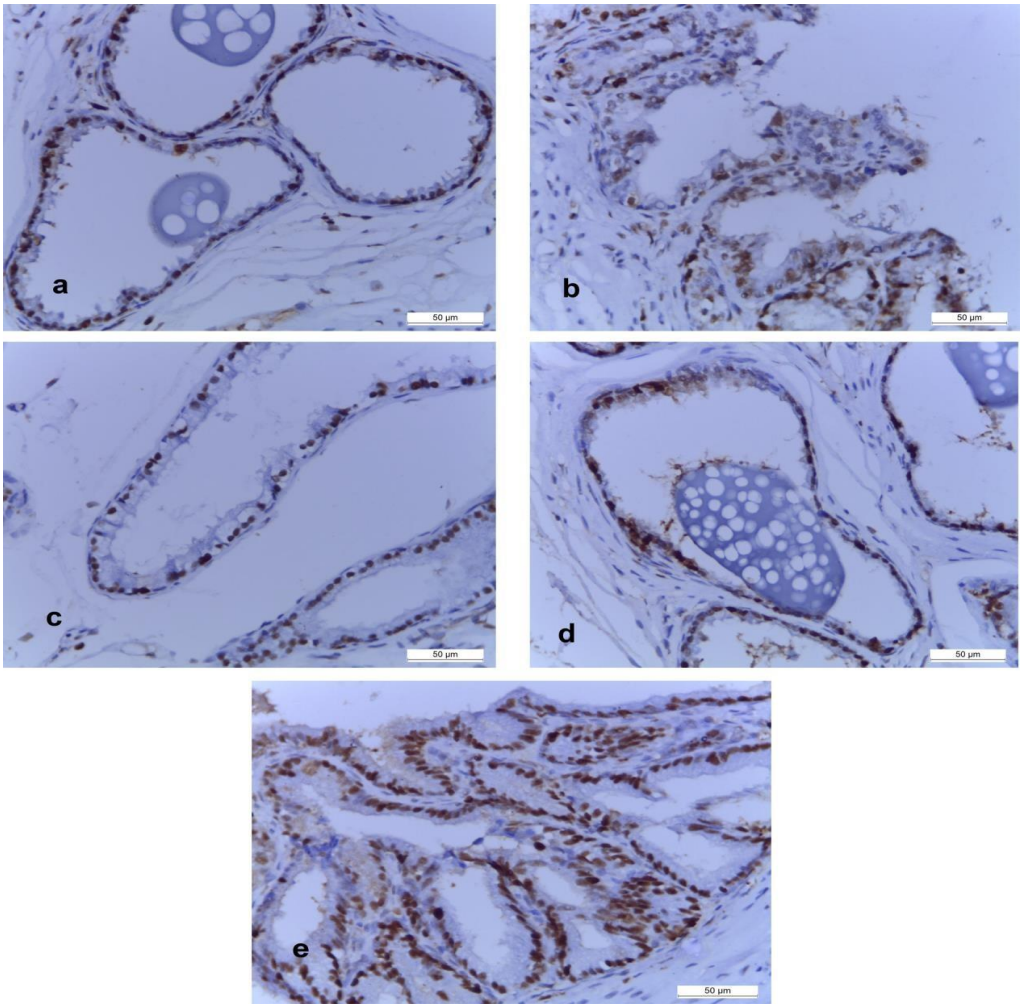

Fig. (5): Photomicrographs of the prostate: a) (group I) showing positive PCNA reaction affecting sporadic nuclei . b) (group II) showing weak positive PCNA reaction . c) (group III), d) (group IV) & e) (group V) showing positive PCNA reaction . (PCNA x 400).

Electron microscopic examination

The prostatic tissue of group I showed clear cell boundaries of most of the acinar secretory cells, and most of the nuclei are apparently normal (Fig. 6a). Group II showed large number of cytoplasmic vacuoles in the acinar secretory cells, with dilated fragmented cisterns of the rough endoplasmic reticulum, the wall of the acinus showed deposition of large amount of collagen fibers (Fig 6b). group III showed shrunken cell membrane of acinar cells with many blebs, some nuclei are shrunken with peripheral condensation of chromatin and the cytoplasm contains large number of vacuoles (Fig6c). Group IV showed shrunken cells, with an irregular cell membrane with many blebs some of the nuclei are shrunken and irregular, the cytoplasm contains large number of vacuoles, the rough endoplasmic reticulum shows a whorl pattern, and the endothelial lining of blood capillaries is atrophied (Fig. 6d). Group V showed large number of microvilli in the luminal border of the acinar secretory cells, the cytoplasm contains many mitochondria, there are many cytoplasmic vacuoles containing electron dense bodies, mostly autophagic vacuoles (Fig. 6e).

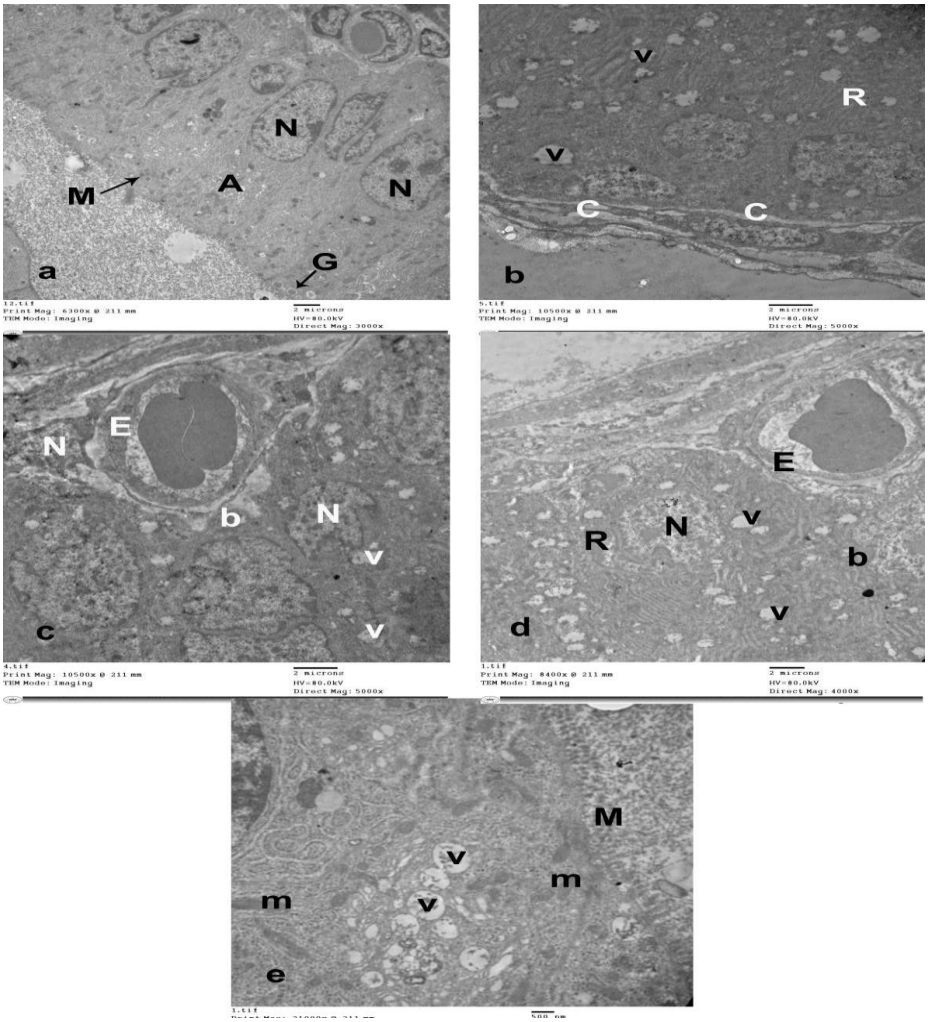

Fig. (6): Electromicrographs of the prostate: a) (group I) showing clear cell boundaries of most of the acina (A). The cytoplasm contains many mitochondria (M) and many secretory granules (G). Most of the nuclei are apparently normal (N). b) (group II) showing large number of cytoplasmic vacuoles (v) , with dilated fragmented cisterns of the rough endoplasmic reticulum (R). The wall of the acinus shows deposition of large amount of collagen fibers (C). c) (group III) showing shrunken cell membrane with many blebs (b). Some nuclei are shrunken with peripheral condensation of chromatin (N). The cytoplasm contains large number of vacuoles (v). Swollen endothelial lining of the capillaries (E). d) (group IV) showing shrunken cells, with an irregular cell membrane with many blebs (b). Some of the nuclei are shrunken and irregular (N). The cytoplasm contains large number of vacuoles (v). The rough endoplasmic reticulum shows a whorl pattern (R). The endothelial lining of blood capillaries is atrophied (E). e) (group V) showing large number of microvilli (M) . The cytoplasm contains many mitochondria (m). There are many cytoplasmic vacuoles (v). (TEM a) x 3000, b), c) x 5000, d) x 4000, e) x 1000).

Histomorphometric results

A significant difference was observed between group II and other groups for the caspase and PNCA expression. Similar results were observed for PCNA protein expression (Table 2).

Table (2): Results of Histomorphometric studies of different groups

	Group I	Group II	Group III	Group IV	Group V
Caspase-3	1.46 ± 0.21	6.87 ± 1.45[a]	2.16 ± 0.56	2.61 ± 0.74	1.32 ± 0.24
PCNA	94.83 ± 8.23	6.45 ± 0.24[a]	91.35 ± 6.92	82.35 ± 5.25	93.39 ± 7.26

[a] statistically significant compared to the other groups ($P < 0.05$).

DISCUSSION

Mesenchymal stem cells have recently gained attention as potent modulators of both the innate and adaptive immune responses and have gained traction in a variety of diseases. MSCs are influential in the treatment of various degenerative diseases and immune disorders [17, 18]. In our study we investigate if the use of the MSCs can add a value in the treatment options of EAP. The MSCs were added as an adjuvant to immunosuppressive agent (tacrolimus) for the treatment of the EAP and the results were greatly promising.

Our results demonstrated that the group of rats treated with MSCS plus tacrolimus showed more obviously better response than the other lines of treatment as indicated by the signs of regeneration observed in the Hematoxylin and eosin examination and electron microscopic picture for the prostatic tissue obtained from all the animals in this group. In that group, most of the acini are within normal appearance, with healthy epithelial lining. There are few corpora amylacea. The fibromuscular stroma is within normal, and with few lymphocytic infiltrates. By Electron microscopy most of the acinar secretory cells (SC) are apparently normal columnar cells with clear cell boundaries and short microvilli on the luminal surface of the cell. The nuclei in most of the cells are apparently normal.

The therapeutic utility of MSCs have been proven in treatment of many prostatic diseases especially the prostatic carcinoma and chronic prostatitis [19,20] and also have been proven in many autoimmune diseases [21-23]. This role may be explained by that the MSCs have an inherent tropism for sites of inflammation. Which is common in prostatitis and their role in modulation of both the innate and adaptive immunity. MSCs have a multitude of immunosuppressive properties through effects on nearly every component of the immune system [24,25]

Two important factors contribute to the success of the MSCs in treatment of EAP first is the ability to traffic to sites of inflammation and to fuse with the inflamed tissue of the prostate through the action of soluble chemokines and cytokines originating from the inflamed cells and the second factor is the immunosuppressive properties of the MSCs [26].

As described before in the literature MSCs can home to sites of prostate cancer and inflammation [27]. MSCs can escape immune surveillance due to the lack of HLA-DR expression and the associated co-stimulatory molecules and so homing and fusion to the cells occur[28]. In our study homing was evident after one week by showing PKH labeled red fluorescent cells within the prostatic tissue (Fig 1). Inflammatory cytokines are secreted by immune and non-immune cells and have a variety of crucial roles in regulating the immune response and mediating the inflammatory response [29] Cytokines act locally as initiators and modulators of immune-induced inflammation [30] In the current study, two inflammatory mediators, TNF-α and IL-1β have been examined as potential indicators in diagnosis and evaluation of suggested treatment of EAP and the reported results revealed that there is a statistical significant high level in the diseased animal group (II) compared to the healthy animal group (I). Yang et al, 2004 and He et al, 2004 have reported the value of TNF-α and IL-1β in prostatic secretions for chronic prostatitis [31,32].

TNF-α, is secreted by macrophages and monocytes and plays an important role in infection and inflammation. Previous studies have reported that the TNF-α levels in seminal plasma are increased in chronic prostatitis patients. TNF-α acts on the prostate endothelial cells under bacterial stimulation increasing the expression of adhesion molecules, leading to the accumulation and infiltration of inflammatory cells. IL-1β is a cytokine that plays a role in the development of prostatitis as it stimulates the prostate gland epithelial cells to produce adhesion molecules and activates leukocytes to release proteolytic enzymes and oxygen free radicals [33,34]. Our results suggested that the expressions of TNF-α and IL-1β can be considered as potential indicators in diagnosis and evaluation of suggested treatment of EAP. Further studies must be implicated to consolidate our results and to prove the role of both inflammatory mediators in EAP.

In our study two distinct diagnostic biomarkers were quantitively measured by histomorphometric measurements to evaluate the response to various treatment lines used in the study. PCNA expression as a biomarker for regeneration and Caspace-3 expression as a biomarker of apoptosis. Our results demonstrated that the group of rats treated by combined therapy (Tacrolimus and MSCs) has a very good prognostic indicators with a high level of PCNA and a very low level of Caspace-3 compared to other groups with a statistically significant level with group II (diseased animals) and with a level very near to the normal control group. PCNA expression has been used in lot of studies as a marker of cell proliferation. Abundant expression of PCNA was found in all animals as an indicator of tissue proliferation [35,36]. Caspases are fundamental components of the mammalian apoptotic machinery. Caspase-3 is a prototypical enzyme that becomes activated during apoptosis in a wide variety of tissues [37].

CONCLUSION

From the present study, it could be concluded that prostatic tissue regeneration in EAP could be achieved by immunosuppressive drugs (tacrolimus). However, MSCs showed even higher promotion of regeneration than tacrolimus therapy. Combined MSCs and tacrolimus was proved to be efficient and reliable mode of treatment of EAP.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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