Potential Role of Prolactin in Modulating the Immuno-Inflammatory Response Induced in a Male Rat Model of Rheumatoid Arthritis

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Abstract

The role of leukocyte-derived PRL as an autocrine or paracrine factor which regulates cytokines production is supported by previous observations. Thus, PRL can influence the immune system directly through up-regulation of its receptors on inflammatory cells.

Objective: Investigating the effect at different prolactin levels and the gene expression of its receptors in modulating the immuno-inflammatory response in a rat model of collagen induced arthritis (CIA), which was induced by a single dose of bovine collagen type II emulsified in complete Freund’s adjuvant injected at the base of each rat’s tail.

Material and Methods: 60 adults normal male rats were divided into Group (I): Normal control group (n=10) vehicle-treated rats. Group (II) CIA group (n=50) rats were randomly subdivided into the following: Group IIA: Untreated group of CIA rats, group IIB: CIA rats treated with metoclopramide, group IIC: CIA rats treated with bromocriptine (BCT), group IID: CIA rats treated with prednisone intake and group IIE: CIA rats treated with a combination of bromocriptine and prednisone. The parameters estimated in the studied groups were plasma levels of TNF-α, IgG and RF the total and differential lymphocytic counts, monocytic count. Also the levels of synovial TNF-α and PRL-R gene expression on peripheral leucocytes and tissue homogenate were evaluated. To further assess the contribution of prolactin on immune response, the plasma and supernatant PRL levels were measured.

Results: A positive strong correlation between PRL levels and arthritic index in CIA animals was clearly apparent when the experimental rats were treated with metoclopramide. On the other hand, when CIA rats were given bromocryptine, clinical and histopathological improvement were observed. The parameters estimated in the studied groups were plasma levels of TNF-α, IgG and RF the total and differential lymphocytic counts, monocytic count. Also the levels of synovial TNF-α and PRL-R gene expression on peripheral leucocytes and tissue homogenate were evaluated. To further assess the contribution of prolactin on immune response, the plasma and supernatant PRL levels were measured.

Conclusion: PRL hypersecretion shifts the balance in the immune response towards higher activity of the immune system cells, which indicated the contribution of PRL to the exacerbation of the disease events.

Key Words: Prolactin - Collagen induced arthritis - Prednisone.

Introduction

RHEUMATOID arthritis is already known to be a common autoimmune disease. Synovial inflammation and hyperplasia are common findings in rheumatoid arthritis that eventually lead to progressive cartilage and bone destruction [1]. In an attempt to elucidate the patho-physiology of rheumatoid arthritis, several current studies supported the concept that chronic inflammation involves T-cell activation [2,3].

All factors played via cytokine-mediated RA pathogenesis reflect the pivotal role of these different cytokines in the disease activity, thus leading to the first clinical trials of a biological therapeutic for RA [4]. These cytokines include mainly tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). Since the enhanced production of these pro-inflammatory cytokines is central to the regulatory role of T-cells as well as macrophages and the induction of an immune response [8].

Furthermore, epidemiologic data as well as the current genetic data clearly reflect a complex genetic component, along with contributions by several hormones [6]. Among these hormones, PRL may be crucial in participating the pathogenesis of the disease [8].

Not only prolactin subserves a biological role related to lactation and reproduction, but it also plays other multiple homeostatic immune actions [7]. Thus, the role of PRL in enhancing the immune ability is receiving a considerable amount of attention as a possible potential therapeutic agent for patients with immuno-deficient diseases [8].

Interestingly, PRL is now identified as a common mediator of an immune-neuroendocrine net-
work, in which these systems communicate with each other. In fact, a great deal of evidence suggests that peripheral lymphocytes and immune-competent cells from the thymus and spleen contain PRL mRNA and release a bioactive PRL quite similar to the pituitary prolactin [9]. Abundant data even suggested that control of this lymphocytic PRL release involves dopamine receptors [10].

Under stressful conditions, PRL is needed to balance the negative effects of glucocorticoids and other immune or inflammatory mediators to maintain steady-state homeostasis. This interpretation is supported by in-vitro studies showing PRL’s protective effect in preventing glucocorticoid-induced lymphocyte cell death apoptosis [11].

In view of these findings, interrelation between the effect of PRL and glucocorticoids in the pathogenesis of autoimmune diseases especially RA has been in the last decade a matter of continuing concern. Since the mechanisms that regulate the immune system appear to be quite complex, and many biological substances, particularly prolactin, seem to be involved [10].

Aim of the work:

The present study was designed to further explore the possible potential role of PRL in the pathogenesis of autoimmune disease through an experimental rat model of rheumatoid arthritis. In addition, this study aimed to detect the interaction between prolactin and glucocorticoids in controlling the mechanisms which participate in the pathogenesis of the disease. Another objective in this study was to investigate the plausibility of using dopamine agonist as a promising therapeutic agent in combating the RA pathogenic events and participating in its treatment of the disease.

Material and Methods

**Experimental animals & groups:**

Sixty male adult albino rats approximately 10-12 weeks of age and of body weights ranging from 150 to 200 grams were included in the study. The animals were placed under ordinary living conditions (e.g humidity, temperature and dark/light cycles) in the Animal house of Faculty of Medicine, Cairo University from Oct-Des 2011. They were kept in wire mesh cages and had free access to food and water. The animals were then randomly divided into the following groups:

1- **Group I: Control (n=10):**

This group represented the placebo group which included the vehicle-treated rats, injected with 0.1 ml isotonic saline in a single dose intradermally at the base of the rat’s tail.

2- **Group II (n=50):**

This group represented the main experimental group in which an experimental model of induced rheumatoid arthritis (adjuvant arthritis) was achieved by intradermal injection of a single dose of collagen type II emulsified in complete Freund’s adjuvant at the base of the rat’s tail. All animals were left for a period of 30 days (which is the time needed for the pathogenesis of the disease [12]).

During this period, the experimental animals in the second group were randomly subdivided into:

**Group II A (n=10):** Represented the control group of rats which received collagen emulsified in complete Freund’s adjuvant.

**Group II B (n=10):** Represented the CIA animals which received dopamine receptor blocker ‘metoclopramid’ in a single dose of 2.2mg/Kg twice weekly by intraperitonial injection throughout the experimental period of the pathogenesis of the disease, to examine the impact of PRL on the events of the disease [13].

**Group II C (n=10):** Represented the CIA animals which received dopamine receptor agonist (bromocriptine) in a daily oral single dose of 10mg/kg (i.e.2mg) using gastric tube [14] to study the effect of bromocriptine as a suppressor of PRL secretion.

**Group II D (n=10):** Represented the CIA animals which received orally a low dose maintenance therapy of prednisone (i.e. 1.35mg) using gastric tube [15].

**Group II E (n=10):** Represented the CIA animals which were treated with a combined therapy of bromocriptine and prednisone, with the same fore-mentioned doses & routes of administration throughout the experimental period of the disease, to study the dual impact of the two drugs on the pathogenesis of the disease.

1- **Study protocol:**

- From day 15 after immunization till the end of experimental period, the rats were examined daily for the onset of clinical manifestations of arthritis [16].
- At the last day of the experiment, before taking of the blood samples the degree of inflammation
was scored by grading each paw from 0 to 4. Based on erythema, swelling and deformity of the joint.

- At the last day of the experiment, immediately before sacrifice, blood samples were withdrawn from the intra-orbital retro-bulbar plexus using a capillary tube and placed in 1 ml Eppendorf tubes. Total lymphocytic count, T-lymphocytes, B-lymphocytes and monocyte counts were evaluated. The plasma was separated by centrifugation and stored at -80°C for assessment of:

Levels of immunoglobulin G (IgG), TNF-α, and Rheumatoid factor (RF) together with prolactin plasma levels and gene expression of prolactin receptors on leucocytes were estimated.

The animals were then sacrificed by cervical dislocation; the knee joints were dissected, cut and separated from the surrounding tissues. Further biochemical analysis was achieved for assessment of: Supernatant prolactin levels in synovial fluid, TNF-α level in synovial fluid and gene expression of prolactin receptors on tissue homogenate. Autopsy samples were taken from the knee joints of rats in different groups and fixed in 10% neutralized formaline solution for 24 hours for histopathological examination.

II- Experimental Procedures:

- Induction of rheumatoid arthritis: Bovine CII (collagen type II) was purified from hyaline cartilage. Male rats (10-12 weeks old) were immunized with a single dose of 100µg of CII emulsified in complete Freund's adjuvant by intradermal injection at the base of the rat's tail.

Preparation of complete Freund's adjuvant: Freund's adjuvant is a solution of antigen emulsified in mineral oil and used as an immuno-potentiator (booster). The complete form, Freund's Complete Adjuvant, (CFA or FCA) is composed of inactivated, dried and heat-killed mycobacterium tuberculosis H37Ra, whereas the incomplete form (IFA or FIA) lacks the mycobacterial components [19].

Preparation of mycobacteria suspension: This protocol describes the preparation of a modified complete Freund's adjuvant (CFA) to be used in rats for induction of adjuvant arthritis. Commercial sources of CFA have not been found suitable for induction of RA, since the particle size of the mycobacteria in the suspension may influence the success of disease induction. For this reason, large particles of mycobacteria had to be broken up by grinding into smaller particles.

Materials and equipment: A dose of 100mg dried, heat-killed mycobacterium tuberculosis (strain H37Ra), 1 ml incomplete Freund's adjuvant (IFA; Difco), pestle, ~7-cm-diameter roughened glass or porcelain mortar and a 15-ml plastic or glass tube.

- Induction of hyperprolactenemia: This was achieved by using metoclopramide HCl, in powder form, purchased from (Sigma-Aldrich Egypt, number C1900). The powder was dissolved in saline, and supplied to each rat in a dose of 2.2mg/Kg twice weekly by intraperitoneal injection throughout the experimental period of the pathogenesis of the disease [13].

- Induction of hypoprolactinaemia: This was achieved by using bromocriptine (BCT), in tablet form, purchased from (Sigma-Aldrich Egypt, number C1900), then was dissolved in saline and given to each animal in a daily oral dose of 10mg/kg (ie 2mg daily) using gastric tube [14].

- Low dose maintenance therapy of glucocorticoids: This was achieved by using prednisone, in tablet form, purchased from (Sigma-Aldrich Egypt, number C1900), then was dissolved in saline and given to each rat in a daily oral dose of 1.35mg [15].

- Scoring of arthritic intensity: The degree of inflammation was scored by grading each paw from 0 to 4. Based on erythema, swelling and deformity of the joint, the grades represent the following: 0 = No erythema or swelling; 1 = Slight erythema or swelling of one of the toes; 2 = Erythema and swelling of more than one toe; 3 = Erythema and swelling of the ankle; 4 = Complete erythema and swelling of toes or fingers and ankle and inability to bend the ankle. All four legs were scored, so that the highest possible score called "arthritic index" which was 16 [17].

The following parameters were measured:

- Determination of immunoglobulin G plasma levels:

  This was achieved by using rat immunoperoxidase assay for determination of IgG.

- Determination of rheumatoid factor (RF):

  This was achieved by using rat rheumatoid factor Elisa kit (RF):

- Detection of plasma and supernatant TNF-α:

  This was achieved by using rat TNF-α immunoassay.
Determination of inflammatory cellular counts:

Total lymphocytic count, T-lymphocytes, B-lymphocytes and monocytic count:

Complete blood count was performed by an automated analyser. The blood was well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counted the numbers and types of different cells within the blood. This is known as the flow cytometry.

Determination of plasma and supernatant prolactin levels:

This was achieved by using Ray bio (R) rat prolactin Elisa kit.

Detection of prolactin receptor gene expression:

This was determined by using real time-PCR (Quantitative Real Time PCR-Protocol) Steps:

Extraction of RNA from whole blood & synovial fluid:

• Total RNA was extracted from whole blood and synovial fluid using SV total RNA isolation system (Promega, Madison, WI, USA).

• Reverse transcription into cDNA:

  The extracted RNA was reversely transcribed into c DNA using RT-PCR kit (Stratagene USA).

• Quantitative real time Polymerase Chain Reaction (qPCR).

Histopathological examination:

After sacrifice, both knee joints were separated with trimming down of the surrounding soft tissues [18]. Autopsy samples were taken from the knee joints of rats in different groups and fixed in 10% neutralized formaline solution for 24 hours. Before the tissue could be fixed and embedded, the specimens were decalcified for 48 hours in 30% formic acid containing 0.28 M sodium citrate, then the knee joints were cut into approximately 2 equal halves in the frontal plane, using the collateral ligament as a landmark [19]. After decalcification, the specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 2pm thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examination through the light microscope (Olympus microscope) using magnification power of 10x40.

The knee joints were evaluated for: Inflammatory cellular infiltration, blood vessels infiltration, bone erosion, cartilage destruction, degree of edema and thickness and intactness of synovial membrane.

Statistical analysis:

Data were statistically described in terms of range, mean and standard deviation (± SD). Comparison of quantitative variables between the study groups was done using ANOVA test (analysis variance) of probability value (p-value) less than 0.05 was considered statistically significant [20].

Results

Effect of induction of rheumatoid arthritis:

The present results have shown a marked significant increase in serum TNF-α, IgG, and RF in the CIA-group II A (p-value <0.01) compared to control group I (vehicle-treated). In addition, the results reported highly significant elevation in the mean values of all studied cellular counts (total lymphocytic count, T-lymphocytic, B-lymphocytic and monocytic counts) in response to induced RA in group II A, when compared to corresponding values of group I (p-value <0.01) Figs. (1,2).

The effect of CIA in group II A at tissue level yielded a significant increase in the mean value of synovial TNF-α and supernatant PRL levels if compared with their corresponding values of the control animals group I (p-value <0.01) Fig. (3).

Furthermore, the current results recorded a highly significant elevation in the mean values of arthritic index in CIA group together with increased plasma prolactin level, compared to that of control rats (group I). In addition there is a significant increase in the mean values of PRL-R gene expression on peripheral blood leucocytes and tissue homogenate (knee joint) in group II A, compared to control group I indicating the stimulatory impact of rheumatoid arthritis on inflammatory cells (p-value <0.01) Figs. (4,5).

Effect of change in the prolactin concentrations on the different parameters of RA: (hyperprolactinemia versus hypoprolactinemia):

When observing levels of TNF-α and IgG in group II B (Induced RA rats treated with metoclopramide) the present results recorded a marked significant increase in their plasma levels compared to group II A (p-value <0.01), thus reflecting the effect of hyperprolactinemia on these parameters. Although there was an increase in the mean value of RF, yet it was without any significant variation (p-value >0.05) when compared to group II A Fig. (6).
Fig. (1): Comparison of plasma levels of TNF-α, IgG and RF in groups I and group II A.

Fig. (2): Comparison of total lymphocytic count, T-lymphocytic, B-lymphocytic and monocytic counts in group I and group II A.

Fig. (3): Comparison of supernatant PRL and synovial TNF-α levels in group I and group II A.

Fig. (4): Comparison of plasma PRL levels and arthritic index in group I and group II A.

Fig. (5): Comparison of PRL-R gene expression on peripheral blood leucocytes and tissue homogenate (knee joint) in group I and group II A.

The values in Figs. (1,2,3,4,5) are represented as mean ± SD.

*: Statistically significant compared to corresponding value in group I ($p<0.0$).
However, on observing levels of TNF-α and RF in group II C (Induced RA rats treated with bromocryptine) the results recorded a marked significant decrease in their plasma levels ($p$-value <0.01), with their compared to group II A and group II B denoting the modulatory effect of decreased prolactin level on these parameters. On the other hand, there was a decrease in the mean value of IgG, but it was without significant variation ($p$-value >0.05) when compared to group II A. However, there was a marked significant decrease in the plasma levels of IgG ($p$-value <0.01) in group II C compared to group II B Fig. (6).

As shown in Fig. (7). In addition, when recording the change in cellular counts (total lymphocytic count, T-lymphocytic, B-lymphocytic and monocytic counts) in group II B marked significant elevation in their mean values ($p$-value <0.01) was detected compared to group II A thus denoting the effect of increased prolactin level on the inflammatory cells. On observing these parameters in group II C the present results revealed a highly significant decrease in their mean values ($p$-value <0.01), compared to both group II A and group II B denoting the modulatory effect of decreased prolactin level on inflammatory cells.

However, the current results revealed a significant increase ($p$-value <0.01) in the mean values of synovial TNF-α and supernatant PRL in group II B compared to the corresponding levels in group II A having, reflecting the effect of hyperprolactinemia on these parameters. On the other hand, our results reported a significant decrease ($p$-value <0.01) in the mean values of synovial TNF-α and supernatant PRL levels in group II C if compared with group II A and group II B, thus reflecting the effect of hypoprolactinemia on these parameters Fig. (8).

When observing the clinical changes in the RA in group II B, there was a significant elevation in the mean values of both arthritic index and prolactin level compared to group II A level. In addition, the current results recorded a significant decrease in the mean values of arthritic index and prolactin in group II C if compared to group II A and group II B. Fig. (9). Our results recorded a significant increase in the PRL-R gene expression on both peripheral leucocytes, and tissue homogenate (knee joint) in group II B, compared to group II A. However, on observing these parameters in group II C the present work recorded a significant decrease ($p$-value <0.01) in their mean values compared to group II A and group II B Fig. (10).
The effect of prednisone treatment versus the combination between prednisone and bromocriptine on different parameters of RA:

Interestingly, the current results recorded a significant decrease ($p$-value <0.01) in the plasma levels of TNF-$\alpha$, IgG and RF in group II D (Induced RA rats treated with prednisone) and in group II E (Induced RA rats treated with bromocriptine and prednisone), compared to group II A. However, the plasma levels of TNF-$\alpha$, IgG and RF in group II E did not show any significant variation ($p$-value >0.05) when compared to group II D Fig. (12).

Furthermore, as shown in figure 13, the present work recorded a significant decrease in the mean values ($p$-value <0.01) of synovial TNF-$\alpha$ and supernatant PRL levels in group II D and in group II E if compared to group II A. However, there was no significant change in their mean values in group II D if compared to group II E. On the other hand, the results demonstrated a significant decrease ($p$-value <0.01) in the mean values of arthritic index, and in prolactin mean value in group II D and in group II E compared to group II A. However there was no significant change in the mean values of arthritic index and prolactin level in group II D compared to group II E Fig. (14).

The present results reported a significant decrease ($p$-value <0.01) in PRL-R gene expression on peripheral blood leucocytes, and tissue homogenate (knee joint) in group II D and in group II E compared to the mean values in group II A. In addition, our results recorded a significant variation ($p$-value <0.01) in PRL-R gene expression on peripheral blood leucocytes in group II E compared to group II D, however there was no significant change ($p$-value >0.05) in PRL-R gene expression on tissues homogenate (knee joint) in group II E compared to group II D in spite of the decrease in its mean value Figs. (16,17).

To further emphasize the relation of prolactin with rheumatoid arthritis index, a correlation study was done, as shown in Fig. (17). This study revealed that prolactin is clearly significantly positively correlated with arthritic index.
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Fig. (12): Comparison of total lymphocytic count, B-lymphocytic, T-lymphocytic and monocytic counts in groups IIA, IID and IIE.

Fig. (13): Comparison of supernatant PRL and synovial TNF-α levels in groups IIA, IID and IIE.

Fig. (14): Comparison of plasma prolactin levels and arthritic index in groups IIA, IID and IIE.

Fig. (15): Comparison of PRL-R gene expression on peripheral blood leucocytes and tissue homogenate (knee joint) in groups IIA, IID, and IIE.

Values in Figs. (11,12,13,14,15) are represented as mean ± SD.
#: Statistically significant compared to corresponding value in group IIA (p<0.01)
$: Statistically significant compared to corresponding value in group IID (p<0.01).
Arthritic index

Fig. (17): Correlation between plasma prolactin levels (ng/ml) and arthritic index among studied groups: Prolactin was strongly positively correlated with arthritic index.

Fig. (18): Gross picture for foot of one of the rats in group I showing normal appearance (no edema and no erythema affecting the ankle joint or the toes).

Fig. (19): Gross picture for foot of one of the untreated CIA rats showing edema affecting the toes.

Fig. (20): Gross picture for foot of one the CIA rats treated with metoclopramide showing edema and erythema affecting the ankle and the toes.

Fig. (16): An agarose gel electrophoresis shows PCR products of PRL-R on peripheral leukocytes (A), PRL-R on tissue homogenate (B) beta actin gene expression (C) in different studied groups.
- Lane M: DNA marker with 100bp.
- Lane 1: PCR products in group I (control).
- Lane 2: PCR products in group II A (induced RA).
- Lane 3: PCR products in group II B (induced RA rats treated with metoclopramide).
- Lane 4: PCR products in group II C (induced RA rats treated with bromocryptine).
- Lane 5: PCR products in group II D (induced RA rats treated with prednisone).
- Lane 6: PCR products in group II E (induced RA rats treated with combined bromocryptine and prednisone).

Fig. (17): Correlation between plasma prolactin levels (ng/ml) and arthritic index among studied groups: Prolactin was strongly positively correlated with arthritic index.
Histopathological Results:

Morphological changes in knee joints in all studied groups:

Fig. (24): Section in the knee joint in group 1 (normal) showing normal bone trabeculi, normal cartilage, normal synovial membrane and no blood vessels infiltration (H and E, x 400).

Fig. (25): Section in the knee joint in group IIA (induced RA) showing: Edema of the joint space and cartilage destruction (H and E, x 400).

Fig. (26): Section in the knee joint in group IIB (induced RA rats treated with metoclopramid) showing thickened synovial membrane denoting chronic inflammation (H and E, x 400). SM: Skeletal muscle.

Fig. (27): Section in the knee joint in group IIC (induced RA rats treated with bromocryptine) showing mild inflammatory cellular infiltration (lymphocytes) (H and E, x 400).
Discussion

The factors triggering RA are thought to be genetic, infectious, environmental and hormonal, which are all involved in complex, inter-related ways. Since interpretation of such generalized data, however, is still obscure and the hormone role in particular is limited, this prompted us to further examine and elucidate in this study the possible impact of PRL in some of the mechanisms involved in these pathogenic events.

To achieve this, an experimental model of RA was applied in which collagen type II (CII) emulsified in Freund’s complete adjuvant (FCA) was given to the experimental animals. This procedure also known as collagen induced arthritis (CIA), which is an autoimmune model that shares the genetic and immunological features with RA (synovial hyperplasia, infiltration of lymphocytes, bone erosion and cartilage destruction) and has therefore been commonly used as an important model of human RA disease [21].

In the present study the collagen-induced arthritis (CIA) has yielded histological and immunological results related to RA, which were observed at the serum as well as the tissue levels (knee joints). Thus, the present findings recorded a significant elevation in the estimated RF, T- and B-lymphocytes in the blood in addition to enhanced total lymphocytic and monocyctic counts. Furthermore, the histological study revealed significant infiltration of lymphocytes at tissue levels associating the synovial hyperplasia.

The results are in accordance with previous reports which concluded that both T- and B-cell activations are crucial in inducing CIA. Indeed, immune mechanisms that include both humoral and cellular immunity to CII have been implicated in the pathogenesis of the disease which was also documented by Kumar et al., in 2004 [22]. The development of CIA is dependant on T-cells mediated activation of auto-reactive B-cells [23]. The major role of B-cells is production of arthritogenic anti-CII antibodies, which is clearly shown by the fact that antibodies reactive with CII can bind to cartilage and induce chronic arthritis [21,24]. This concept was further confirmed by our demonstration of significantly increased IgG production in the serum of CIA animals.

Experiments have identified these specifically activated arthritogenic antigenreactive T–lymphocytes to infiltrate the synovium and synovial fluid thus initiating the harmful response [25]. In fact, additional evidence revealed that these T-cells (Th1) are antagonized by the consistent and long-lasting suppressive effect of Th2-induced-secretion of IL-4, IL-10 and IL-13 [23]. These lymphokines are well known to inhibit several CIA-related pro-inflammatory cytokines including TNK-a and IFN-y. Thus, it was concluded that the preclinical phase of a CIA model mimics in human the Th1/Th2 imbalance which features RA [21].

Prolactin, which has been known to possess various roles in reproduction and lactation, has also been confirmed to have an immuno-stimulatory effect. This attempted us to further investigate this PRL impact in RA [26]. In studies related to multi-organ and organ-specific autoimmune diseases including SLE, Hashimoto’s thyroiditis, and multiple sclerosis, hyperprolactinemia has been commonly observed [27]. This revelation was further emphasized in our experiments in CIA rats in which
significantly increased levels of endogenous PRL were demonstrated.

This observed increase in the endogenous PRL serum levels in response to CIA was described in several systemic as well as organ-specific autoimmune diseases to result from several factors. These include an increased release of PRL from the anterior pituitary due to the release of inflammatory cytokines in the circulation or may possibly be due to reduced production of suppressive dopamine. In addition, a most convenient explanation reveals that the increased production of prolactin which may associate RA is produced by the immune system cells in particular T- and B-lymphocytes [28].

The increased PRL levels recorded in the present work after administration of metoclopramide in RA rats, confirm this concept which was reflected in the significant elevated levels of IgG and TNF-α both in plasma and synovial fluid which lead eventually to exacerbation of the pathogenic events. An earlier study explained the increase in the antibodies and TNF-α production in RA to be mainly due to a direct consequence of high serum PRL levels [29]. The increased PRL was concluded in these results to produce an excessive inflammatory response initiated by infiltration of macrophages to the host tissues. Even more, it was also suggested that upon treating the murine peritoneal macrophages with PRL, TNF-α and nitric oxide production was induced [29]. It is thus, hypothesized that PRL treatment triggers Ca ++ signaling and mediates NO production via stimulation of protein tyrosine kinase, and mitogen-activated protein kinase (MAPK) pathways [30,31].

The significantly increased IgG production which has been particularly identified in the present study after supplying the CIA rats with metoclopramide, is thought to be due to enhanced B-cell functions induced by the increased levels of PRL, this was also confirmed by Terasaki et al., In 2010 [32]. Furthermore, the presently estimated levels of RF in the CIA animals, which is IgM antibody in nature, emphasizes the previous observations that PRL enhances mature B-cell functions by increasing IgG and IgM antibody production as well [27,29].

This positive correlation between PRL levels and the arthritic index in CIA animals was clearly apparent in the experimental rats that were treated with metoclopramide and yielded significantly high PRL levels. On the other hand, when CIA rats were given the dopamine agonist (bromocryptine), the present results showed significant clinical and histopathological improvement which emphasizes the impact of the high PRL levels on the pathogenic events and the activity of RA.

These results are supporting to those of previous studies which concluded the existence of this correlation between PRL and immune regulation [33,34], since hypoprolactinemic animals were shown to be deficient in mounting B- and T-cell-mediated immune responses this was documented by Eijsbouts et al., 2005 [33]. When injection of PRL or other lactogenic hormones was given, the immunno-competence was restored to these animals.

The fact that other studies established the production of PRL by rheumatoid synovial T-cells in humans [21], this confirms the present results and explains in this regard the significantly increased PRL levels in supernatant synovial fluids of CIA rats as well as those treated with metoclopramide. In particular, the high PRL levels assessed in the present results in the supernatant fluid of RA rats may explain the increased levels of TNF-α depicted in the synovium. This potent potential role of PRL on the pathogenesis of the disease was further confirmed by the current study to be mediated by significantly increased levels of PRL-R expression on peripheral leucocytes as well as on the tissue homogenate.

The present results confirmed that when the CIA rats were treated with BCT, in which a significant decrease in the levels of IgG, RF and TNF-α was observed both in the plasma and synovial fluid. These findings emphasize the studies which postulated that BCT mediates the suppression of immunoglobulin levels [35]. Bernichtein et al., 2010 [36] also reported a significant decline in the total and differential lymphocytic counts as well as decreased levels of monocytic count. This was explained by Keith and his colleagues in 2007 [38], to be due to the reducing effect of BCT on IFN-γ production and T cell-dependent killing of microorganisms by macrophages.

It is worthy to mention that the dose of BCT used in this study was convenient and agreeable with the relevant study for inducing hypoprolactinemia but not to normalize PRL levels [14]. Also, this dose was effective in the decreasing significantly the signs of local joint inflammation and lymphocytic infiltration of the affected joints in CIA rats.

Furthermore, the present results showed that BCT treatment did not only decrease PRL levels in the serum and synovial fluid, but also caused a
significant suppression in the gene expressed levels of PRL-R on lymphocytes (T- and B-lymphocytes) and monocytes. It is relevant to mention that hyperprolactinemia induced in animals by BCT has also been shown to induce impairment of lymphocyte proliferation and macrophage-activating factor production [37].

In the present experiments, BCT improved the signs of adjuvant arthritis which confirms a number of in-vitro and in-vivo studies in which the abnormalities in T-cell function and proliferation by hyperprolactinemia were corrected with BCT administration. Furthermore, the associated neutrophil chemotaxis was decreased together with inhibition of NK cells and the activity of APCs [32]. The current work also provides evidence that prednisone treatment resulted in decreased TNF-α, PRL levels and down-regulation of its receptors in rats with RA. In previous studies, higher PRL/cortisol ratio was observed, with higher serum levels of IL-1β and TNF-α [34]. In particular, these observations were recorded during nocturnal hours in which there was an imbalance in favor of proinflammatory hormones as opposed to the levels of anti-inflammatory hormones [33].

The reduced total and differential lymphocytic counts by prednisone treatment was accompanied with decrease in IgG production which is contributed to direct lymphocytic depletion and B-cell death by encouraging apoptosis or direct cytolysis [38,39]. It is becoming clear that under stressful conditions, PRL is needed to balance the negative effects of glucocorticoids and other immune or inflammatory mediators to maintain steady-state homeostasis by inhibiting glucocorticoid-induced T-lymphocyte apoptosis [32].

Thus, excessive PRL production in RA and insufficient cortisol secretion for the degree of inflammation may lead to exacerbation of the proinflammatory state which might play a role in RA pathogenesis [40]. This concept may also explain the resistance and sensitivity to endogenous glucocorticoids that may associate with rheumatoid arthritis.

Several mechanisms may be involved in resistance and susceptibility to glucocorticoids, including reduction of the number of glucocorticoid receptors and/or a reduced affinity for the ligand, polymorphisms in GR genes, together with hyper-expression of GR-β that modify the glucocorticoid response by inducing intrinsic glucocorticoid resistance [41].

The present results, which revealed a decreased plasma level of PRL following prednisone intake which confirms the suppressive effects of glucocorticoid on PRL secretion in both pituitary and nonpituitary cell lines as well as inhibition of lactotroph differentiation in rats was previously documented by Yazid et al., 2009 [42].

**Conclusion:** Positive correlation between the levels of PRL and the RA activity exists that may create a significant potential impact on the pathogenic events of the disease in patients. The full understanding of the molecular basis of PRL action in RA patients may lead to the development of more rational therapeutic strategies to test the efficacy of PRL-lowering agents as an adjunctive therapy in the management of such debilitating disorders.

**Recommendations:** The prolactin levels should be checked in patients with persistently active RA despite their treatment, since coincidental hyperprolactinaemia of pituitary origin may be detected in some of RA patients since, of prolactin inhibitor to their treatment in particular bromocriptine with its ability to decrease both peripheral and pituitary PRL production can represent a useful adjunctive therapy in certain RA patients particularly those with refractory disease or in patients who suffer from sever side effects due to chronic use of glucocorticoids.

**References**


8- BOUCHARD B., ORMANDY C.J., DI SANTO J.P. and KELLY P.A.: Immune system development and function


