STUDY OF CORRELATION BETWEEN INTERLEUKIN-1 AND RESPONSE OF NEPHROTIC SYNDROME TO STEROID THERAPY

A THESIS

Submitted for the Partial Fulfilment of
The M.D. Degree in (Internal Medicine)

Presented By

MONA HOSNY ABD EL SALAM
M.B.,B.Ch., M.Sc. (Internal Medicine)

Under Supervision Of

PROF. DR. WAHID MOHAMED EL-SAID
Professor of Internal Medicine & Consultant Nephrologist,
Faculty of Medicine, Ain Shams University.

PROF. DR. MOHAMED ALY IBRAHIM
Assistant Professor of Internal Medicine,
Faculty of Medicine, Ain Shams University.

PROF. DR. SHADIA MABROUK
Professor of Pathology,
Faculty of Medicine, Ain Shams University.

PROF. DR. MONA RAFIK
Professor of Clinical Pathology,
Faculty of Medicine, Ain Shams University.

Faculty of Medicine
Ain Shams University
1995

ACKNOWLEDGEMENT

I would like to thank ***Prof. Dr. WAHID MOHAMED EL-SAID,*** *Professor of Internal Medicine and Consultant Nephrologist, Faculty of Medicine, Ain Shams University, for his great help and support.*

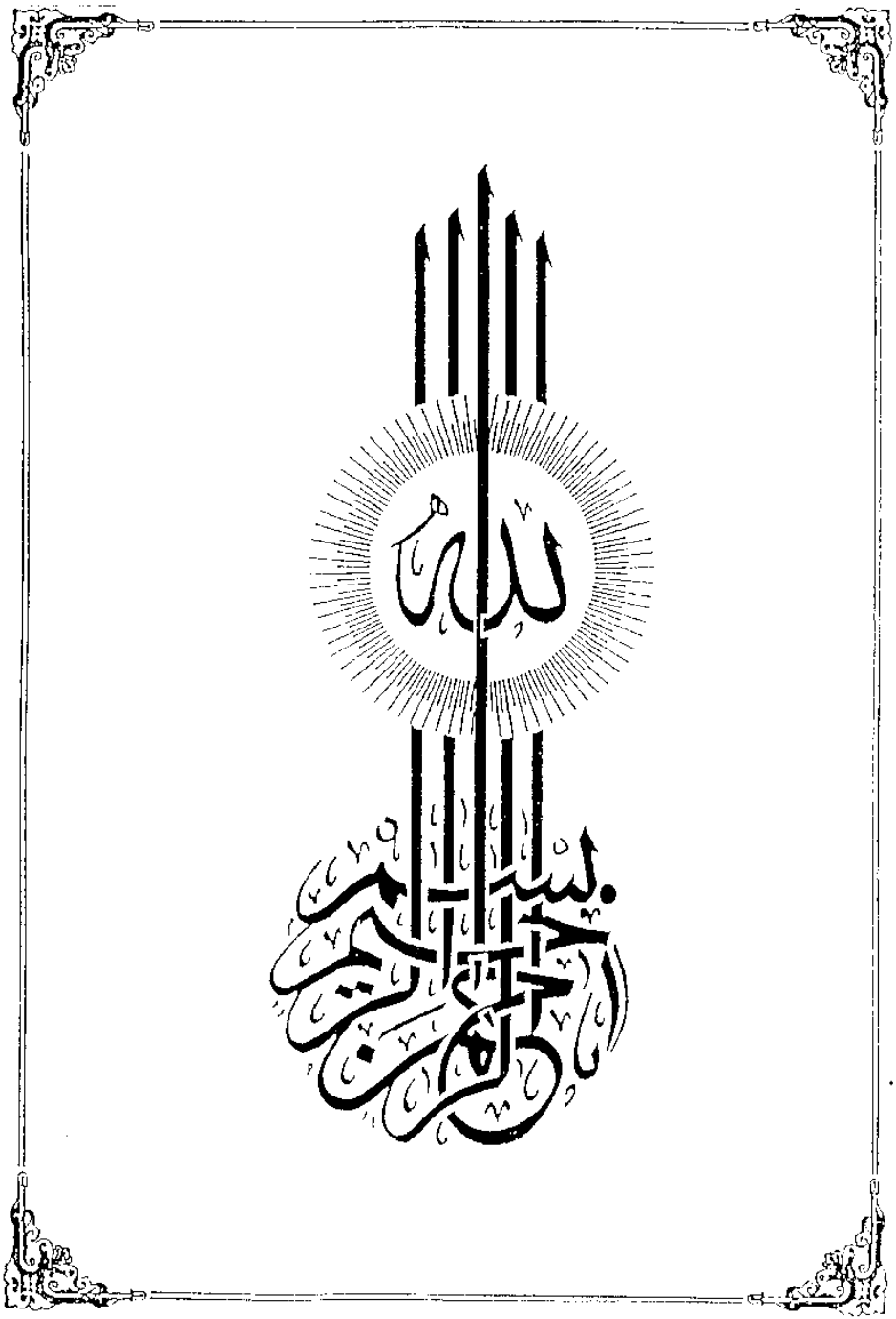
Also, I would like to thank ***Prof. Dr. MOHAMED ALI IBRAHIM,*** *Assistant Professor of Internal Medicine, Faculty of Medicine, Ain Shams University, for his great effort in this work.*

I feel great gratitude to ***Prof. Dr. SHADIA MABROUK,*** *Professor of Pathology, Faculty of Medicine, Ain Shams University.*

Also, I would like to express my gratitude to ***Prof. Dr. MONA RAFIK,*** *Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University.*

I feel grateful to ***Prof. Dr. HANY REFAAT, Dr. SHADIA BARAKAT*** *and* ***Dr. MAHY EL-TEHEWY,*** *for their support.*

I offer special greetings for ***Dr. MAHA EL-GAAFARY,*** *Community Department, Faculty of Medicine, Ain Shams University, for her great help. Without her effort, this work would not be possible.*



CONTENTS

Introduction And Aim Of The Work

INTRODUCTION
AND AIM OF THE WORK

In spite of the wide classifications of the histopathological studies in nephrotic syndrome, no one can predict which type of nephrotic patient will respond to steroid leading to blind clinical trial and error of steroid therapy in most of them. Moreover, renal biopsy is not feasible, practical or contra-indicated in many cases of N.S. A search for other markers that can withstand the use of renal biopsy, including a marker for diagnosis and prognosis is needed ***(Schnaper et al., 1985).***

Interleukin-1 (IL-1) derived from macrophages is responsible for proliferation of cytotoxic T-cell (a step which is blocked by corticosteroids) ***(Aune et al., 1984).***

IL-1 seems to be non-specific and mediates several components of the systemic acute phase response. It affects several non-leukocytic targets such as the liver, pancreas, bone, cartilage, muscle, synovial fibroblasts and brain ***(Dinarello, 1984).***

Thus, the aim of this work is to estimate interleukin-1, which is blocked by steroid, in nephrotic syndrome patients with different histo-pathological types before and after steroid treatment correlating the results with clinical and laboratory data.

Review Of Literature

INTERLEUKIN-1

Production of IL-1 :

Interleukin-1 was originally defined as a lymphocyte-activating factor (LAF) produced by monocytes or macrophages ***(Aarden, 1979).***

Stimulants of IL-1 :

The discovery of IL-1 activity has provided support for the hypothesis of ***Allison and Davis (1971),*** that adjuvants affect the immune system by stimulating macrophages to produce and release T-lymphocyte activating mediators, many of the agents which increase IL-1 production are well known adjuvants, such as lipopolysaccharides (LPS), or components of mycobacteria (PPD) and muramyl dipeptide ***(Gery and Waksman, 1972).***

Mode of action of IL-1 :

An elevation in cyclic GMP was found by ***Katz et al., (1978)*** in rat lymphocytes incubated with rat IL-1.

IL-1 may increase the number of antigen binding sites and change membrane fluidity on murine lymphocytes ***(Lonai and Steinman, 1977).*** The major pathway by which IL-1 may serve as a mediator of the immune response seems to be related to its capacity to stimulate the production of IL-2 by T-lymphocytes. This function was suggested by ***Farrer and Koopman (1979)*** and was established by other studies.

Studies have indicated that the expression of IL-1 effect on lymphocytes depends essentially on its capacity to induce IL-2 production. IL-2 is assumed to mediate the expansion of lymphocyte clones following antigenic stimulation ***(Gilles et al., 1980).***

Relationship between production and release of IL-1 :

Studies have indicated that, extracellular IL-1 activity and the total IL-1 production are not necessarily directly related. The first demonstration of a dissociation between the production and release of IL-1 was reported by ***Unanue and Kiely (1977)*** by unstimulated cultures of murine macrophages.

IL-1 is known to play an important role in immunoregulation and inflammation. This mediator has been shown to have a broad spectrum of *in vitro* as well as *in vivo* activities. Accordingly, IL-1 not only activates thymocytes and lymphocytes but also stimulates granulocytes, fibroblasts, osteoblasts, hepatocytes, smooth muscle cells and may cause fever ***(Dinarello, 1984).***

IL-1 has been known by many names, each one reflecting a different biological property, for example, it had been described for its ability to produce fever (endogenous pyrogen activity), induce acute phase changes (leukocytic endogenous mediator), stimulate synovial cell prostaglandin E_2 and collagenase release (mononuclear cell factor), augment mitogen and antigen responses in B-cells (B-cell activating factor), activate T-cell and induce IL-2 and IL-2 receptors (lymphocyte activating factor), induce cartilage breakdown (catabolism), initiate muscle proteolysis (muscle

proteolysis inducing factor) and stimulate bone resorption (osteoclast activating factor) ***(Dinarello, 1986).***

Structure and molecular biology :

IL-1 is a protein factor.

In late 1984, two forms of IL-1 had been cloned, and the complete amino acid sequences were described. Cloning of two distinct human IL-1 termed IL-1 α and IL-1 β have been reported ***(March et al., 1985).***

IL-1 α is hematopoietic and potentiates the proliferation effect of GM-CSF on immature bone marrow. IL-1 β protects granulocytopenic mice against lethal Pseudomonas infection ***(Furutani et al., 1985).***

Monoclonal anti-IL-1 :

Several heterologous antisera which bind to different IL-1 molecules and block their biological activities have been described ***(Mizel et al., 1983).***

Koch et al., (1986) were able to develop a monoclonal antibody which neutralizes IL-1 activity and bind to both of the human IL-1's.

Moreover, it has been shown that the antibody was also capable of blocking the biological activity of murine IL-1 ***(Luger et al., 1986).***

There are similar antigenic determinants present on both IL-1 α and IL-1 β and the region responsible for the biological activity of IL-1

molecules appears to be located close to this part of IL-1 molecule. The hypothesis that IL-1 α and IL-1 β share a common active site is also supported by a finding showing that apparently there is only one high affinity plasma membrane receptor for both IL-1 α and IL-1 β ***(Dower et al., 1985).***

Effect of IL-1 on immunocompetent cells :

IL-1 elaboration is important in B and T-lymphocyte differentiation and proliferation, lymphokine elaboration, and antibody production ***(Thiele and Lipskey, 1982).***

Prostaglandins as endogenous mediators of IL-1 production :

The production of IL-1 by phagocytic mononuclear cells may be a fundamental event in the initiation and maintenance of the immune responses ***(Kunkel et al., 1986).***

IL-1 causes the release of prostaglandins from a variety of cells, including fibroblasts, chondrocytes, monocytes and vascular endothelial cells ***(Dinarello et al., 1983).*** Indeed, many of the activities of IL-1, such as fever and inflammatory activities, are linked to stimulation of PGE_2 release ***(Goodwin and Ceuppens, 1983).***

Kunkel et al., (1986) and other investigators reported that PGE_2 inhibit expression of IL-1 activity by the monocyte and suggested that IL-1, as classical hormones, can regulate its own production through a self-induced inhibitor PGE_2.

Prostaglandins exhibit suppressive effects on several steps of immune responses *in vitro* ***(Goodwin and Ceuppens, 1983).***

The inhibitory effect of PGE_2 on IL-1 expression may indirectly influence the T-cell as IL-1 augments expression of IL-2 activity ***(Knudsen et al., 1986).***

Role of interferon-γ and α in IL-1 synthesis and secretion :

IL-1 is produced in the body primarily by mononuclear phagocytes. ***Mayernik et al., (1984)*** demonstrated that human blood monocytes cultured *in vitro* differentiated into macrophages and lost the capacity to secrete IL-1 in response to endotoxin lipopolysaccharides (LPS).

However, ***Mag et al., (1985)*** reported that IL-1 synthesis and secretion of human *in vitro* macrophages were profoundly influenced by IFN-γ. Addition of IFN-γ re-induced IL-1 secretory potential, while withdrawal of IFN-γ at anytime from cultures resulted in gradual loss of macrophage IL-1 secretory capacity. In contrast to the distinct effect of IFN-γ, the role of IFN-α in IL-1 synthesis and secretion is not clear.

Interleukin-1 as a cytokine inducer :

Billiau (1987) showed that human IL-1 has the potential to induce human interferon-β and a granulocyte-macrophage stimulating factor (GM-CSF).

The IFN-β-inducing effect was confirmed with purified preparation of recombinant-DNA-derived preparation of both IL-1 α and IL-1 β.

Some of the activities of IL-1 are shared by other cytokines. The previous experiments emphasize the possibility that some of these resemblances between IL-1 and other cytokines may be due to ability of IL-1 to induce these substances in the assay system used ***(Bellanti and Rocklin, 1985).***

Multiple biological activities of recombinant IL-1 :

The two forms of IL-1 have been expressed in bacterial and mammalian cells, and recombinant forms of IL-1 were made available. Recombinant IL-1 of either form induces the same broad spectrum of biological responses which had been reported for IL-1's purified from cuptured cells ***(Dinarello, 1985).***

Biological activities of recombinant human IL-1 (Dinarello, 1986):

In vivo :

- Fever in mice, rats, rabbits and guinea pigs.
- Hypozincemia, hypoferremia.
- Neutrophilia.
- Slow wave sleep induction.
- Hepatic acute phase protein synthesis.
- Increased survival rate in immunosuppressed mice.
- Increased bacterial clearance in immunosuppressed mice.

- Increased cortisone levels in mice and rats.
- Increased ACTH levels in mice and rats.
- Accumulation of neutrophils in tissues.

In vitro :

- Chemotaxis of lymphocytes, monocytes.
- Increase IL-2 receptors and increased IL-2 production.
- Synergism with IL-2 in natural killer cell assay (Human).
- Proliferation of dermal fibroblasts.
- Induction of fibroblast and endothelial GM-CSF activity.
- Induction of endothelial cell procoagulant activity.
- Induction of human endothelial cell neutrophil adhesion.
- Production of PGE_2 in dermal and synovial fibroblasts.
- Production of PGI_2 in human endothelial cells.
- Decreased hepatocyte albumin synthesis.
- Increased neutrophil and monocyte thromboxane synthesis.
- Degranulation of human basophils (histamine release).
- Cytotoxic for human melanoma cells.
- Cytotoxic for human beta islet cells (insulin producing).
- Increased collagenase from human synovial fibroblasts.
- Increased collagenase from chondrocytes.
- Increased bone resorption.

Some effects of IL-1 :

Acute phase response :

One of the primary responses to infection, injury or immunization, is a pronounced local inflammatory response in which the accumulation of mononuclear phagocytes rapidly takes place. There are widespread metabolic and endocrinological abnormalities in human undergoing such a response. Together, these changes are frequently termed acute phase response. It is now clear that many components of the systemic response, both clinical as well as laboratory changes, are due to the actions of IL-1 on several target tissues. These include systemic symptoms such as fever, generalized myalgia and arthralgia, headache, lassitude and even sleepiness ***(Dinarello, 1986).***

One of the most dramatic effects of IL-1 is its ability to induce hepatocytes to synthesize a spectrum of acute phase proteins, these include C-reactive protein, complement components and various clotting factors. At the same time, albumin levels appear to decrease. IL-1 also regulates the transcription of collagen leading to increased synthesis ***(Canalis, 1986).***

Pain :

IL-1-inducing prostaglandin E_2 (PGE_2 from muscle, fibroblast, and synovial cells suggests that pain symptoms are likely initiated by increased levels of IL-1 ***(Dayer et al., 1986).***

Sleep disturbances :

Sleep disturbances have been reported in patients during various