

Serum Interleukin-8 and Hs-C Reactive Protein Determination in Iraqi Multiple Sclerosis Patients: Case Control Study

Khawla A. A. AL-Sammariey

ABSTRACT:

BACKGROUND:

Multiple sclerosis (MS) is an autoimmune disease caused by a complex disorder that cluster many common diseases in children and adults, leading to chronic inflammatory disease of the central nervous system with morphological hallmarks of inflammation, demyelination, axonal loss and gliosis. Factors responsible for MS and its progression are still not fully understood, but it has been suggested that both environmental determinants and genetic susceptibility are involved.

OBJECTIVE :

The aim of this study is to evaluate the role of inflammatory markers ;hs-CRP and IL-8 in pathogenesis of MS and to see the correlation between them.

PATIENTS AND METHODS:

Twenty one MS Patients (7male,14 female) attending the MS Clinic at Baghdad teaching hospital, their ages range between(20-32) years (15) age- and sex- matched healthy control participated in the study. Investigations included serum measurements of high sensitive C-reactive protein (hs- CRP) and interleukin-8 in patients and control groups.

RESULTS:

The result of the present study showed significant increase in both CRP($p < 0.001$) and IL-8 ($p < 0.001$) in the serum of MS patients compared to control.

CONCLUSION:

There is evidence that neuroinflammatory process due to elevation of CRP and IL-8 has an important role in MS .

KEYWORDS: hs-CRP,IL-8,Multiple sclerosis.

INTRODUCTION:

Multiple Sclerosis (MS) is a chronic autoimmune inflammatory disorder of the central nervous system (CNS) ⁽¹⁾. MS is a neurological disease that occurs when the insulating material i.e., myelin around nerve cells in the central nervous system is damaged. It is caused by autoimmune reactivity of T cells towards CNS myelin components. MS progression inevitably leads to the loss of motor function, sensitive disturbances and cognitive impairment because of the immune mediated demyelination and axon degeneration. MS is one of the most common neurological disorders, which mainly affects young adults ⁽²⁾, and causes gradual decrease of their quality of life ⁽³⁾. Infectious organisms, most likely viruses, have long been a suspect for triggering the autoimmune response in people genetically susceptible to MS ⁽⁴⁾.

The causes of the disease and its severity are completely unpredictable and remains a mystery⁽⁵⁾. Many different etiologies have been suggested to play a role in the pathogenesis of MS, including exposure of genetically susceptible individual to an environmental factors during childhood, e.g. virus, which eventually leads to an immune-mediated inflammatory demyelination⁽⁶⁾. Reactive species have also been implicated in the pathogenesis when white matter disorders have been implied ,such as in MS ⁽⁷⁾.

C-reactive protein (CRP) is a member of the class of acute-phase reactant, as its levels rise dramatically during inflammatory processes occurring in the body. This increase is due to a rise in the plasma concentration of some cytokines, which are produced predominantly by macrophages as well as adipocytes⁽⁸⁾. CRP is thought to assist in complement binding to foreign and damaged cells and enhance phagocytosis by macrophages, which

Department of BioChemistry College of Medicine.
Baghdad University.

express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infection ⁽⁹⁾.

Interleukin-8 (IL-8) is among cytokines, which are substances primarily produced by macrophages and monocytes during the inflammatory process. It stimulates acute phase proteins production since cytokine production and the balance of pro inflammatory/ anti inflammatory cytokines are controlled by both immune system ⁽¹⁰⁾ and the central nervous system ⁽¹¹⁾.

The aim of the present study was to investigate the circulatory concentration of the pro inflammatory cytokine IL-8, and an example of acute phase protein (CRP) in MS patients, and to compare the values of these two parameters with those obtained from healthy controls.

PATIENTS AND METHODS:

The study was carried out on (21) MS patients (7male,14female) attending the multiple sclerosis clinic, medical city in Baghdad/Iraq, (which is the only specialized clinic for treating MS patients in the country), in addition to ⁽¹⁵⁾ healthy controls. Their ages ranged between 20-32 year .

All MS patients are diagnosed according to clinical criteria including electrophysiological test (Evoked potentials, spinaltap), cerebrospinal fluid analysis, and neuroimaging (MRI) test . The diagnosis was performed by a specialist neurologist at the center. All patients had a relapsing-remitting course of MS (RRMS). All patients were on treatment with β interferon (44 μ g) given subcutaneously three times weekly. More than 3ml venous blood was taken from each individual in both MS patients and control group using disposable plastic syring 21G

needle. The blood was placed in plane tubes, left for 15minute at room temperature, centrifuged at 2500rpm for 10min to separate serum, which was stored at-20 $^{\circ}$ C unless used immediately.

Determination of CRP:

It was performed using a ready kit for hsCRP ELISA from DRG CRP, hs (C-reactive protein EIA-3954) which is based on the principle of a solid phase enzyme-linked immunosorbent assay.

Determination of IL-8:

Using ELISA kit from Biosource Europe S.A.Compary, Belgium. The base cytokine (ELISA) sandwich type make use of highly purified anti cytokine monoclonal antibodies (captured anti bodies), which were non covalently adsorbed (coated) onto plastic micro well plate. The immobilized antibody serve to especially cytokine protein (IL-8) present in the sample which is applied to the plate.

Statistical analysis:

The data of the present study were expressed as mean \pm standard deviation (mean \pm S.D) using student t-test depending on the p-value \leq 0.05 considered statistically significant.

RESULT:

Table1 shows the (mean \pm SD) values of serum hs-CRP and IL-8 levels in patients with MS and healthy controls. The (mean \pm SD) of serum hs-CRP of MS patients was significantly increased compared with that of healthy controls (9.875 \pm 1.7ng/ml ,5.14 \pm 0.828ng/ml ;p<0.001). Also, serum value (mean \pm SD) of IL-8 level was significantly elevated in patients with MS in comparison with healthy controls (129.11 \pm 17.78ng/ml,79.83 \pm 7.34ng/ml) ; p<0.001).

Table 1:Mean(\pm SD) values of serum levels of IL-8 and hs-CRP levels in patients with MS and healthy controls.

parameter	MS-patients (n=21)	Control (n= 15)
hs-CRP (ng/ml)	9.875 \pm 1.7	5.14 \pm 0.828
IL-8 (ng/ml)	129.11 \pm 17.78	79.83 \pm 7.34

DISCUSSION:

The present study reports for the first time in Iraq on investigation of IL-8 and CRP in patients with MS. A significant elevation in serum hs-CRP levels in MS patients group compared to controls may be attributed to the fact that hs-CRP has both proinflammatory and anti- inflammatory action. Pro-inflammatory effect is through induction of inflammatory cytokine and tissue factor for shedding IL-receptors by monocytes ⁽¹⁴⁾.

However, the primary effect of CRP may be anti-inflammatory by diminishing the accumulation of neutrophils at inflammatory sites by reducing their adhesion to endothelium⁽¹⁵⁾.

These results are in agreement with other studies ⁽¹⁶⁾ who claimed that since MS is an autoimmune inflammatory disease of CNS, the pathogenic T-cells which were permitted by macrophages for infiltration of CNS will initiate a cascade of pro-

inflammatory cytokines, which activate microglia, recruit additional inflammatory cell including macrophages, leading to further inflammation⁽¹⁷⁾. Since interleukine 8 (IL-8) is the major chemoattractant of neutrophils; it mediates changes in neutrophil shape, transendothelial migration, induces exocytosis of granule enzymes such as elastase and myeloperoxidase and a respiratory burst where hydrogen peroxide and superoxide radicals are produced⁽¹⁸⁾.

CONCLUSION:

Could be drawn from the present results, which suggest that neuroinflammatory processes, and especially inflammatory mediators such as IL-8, also have an important role in MS. Further studies are needed to elucidate at least in part the role of IL-8, especially in evaluation of treatment strategy based on cytokine results in patients of MS at different stages.

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