Estimation of Anti-intrinsic Factor IgG and High Sensitivity C- reactive Brotein Levels in Patients With Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) that is increasingly recognized worldwide in children and adolescent; beside genetic factors, inflammatory processes could potentially play an important role in the pathogenesis of MS like high sensitive C reactive protein (hs-CRP). Intrinsic factor (IF), which is a glycoprotein produced by the parietal cells of the stomach is necessary for the absorption of vitamin B12 later in the small intestine. Since MS and vitamin B12 deficiency is frequently misdiagnosed as MS, this study focused on the assessment of hs-CRP and anti-intrinsic factor IgG antibodies in MS patients. Fifty-two MS patients have been receiving IFN-beta were enrolled as a target group and 30 age and sex matched cases were considere as a control group. The level of hs-CRP and anti-intrinsic factor IgG antibodies were investigated by ELISA. We found from a total of 52 MS patients, 27 were females (51.9%) and 25 were males (48.1%) with a mean age 36.36 years and mean of the duration of the disease being 5.33 years. We did not observe any differences in hs-CRP levels between MS patient and healthy controls (p>0.05); while there was a high significant increase in the level of anti-intrinsic factor antibodies in MS patients' sera compared with control group (p<0.05). Both serum hs-CRP and anti-IF antibody levels are not related to the age of patients who had the onset of MS under or over 18 years of age (p>0.05). On the other hand; there was a statistically significant correlation between the levels of anti-IF antibody and the duration of the disease (p<0.05). The results suggest that MS patients have other autoimmune diseases that mimic MS symptoms and played a certain role in the pathogenesis.

Introduction

Multiple Sclerosis (abbreviated MS, also known as disseminated Sclerosis or encephalomyelitis disseminate) is an inflammatory disease of the central nervous system(CNS) that target the myelin sheath surround nerves and myelin –producing oligodendrocytes leading to demyelination and scarring as well as a broad spectrum of sings &symptoms (Alizadeh and Babron,2003 and Sharma et a,l,2010). The name
multiple sclerosis means "many scars and refers to all the scars that the illness causes in the CNS that is increasingly recognized world wide in children and adolescent (Eliaeson, et al., 2008). This disease was first described in 1835 by Jean Martin Charcot (Mohammed et al., 2009). The prevalence of MS is approximately 60 cases per 100000 and the incidence is 3 cases per 100000 per year (WHO, 2006); but these statistics vary considerable between countries; in Iraq, MS is not rare; its demographic and clinical data, in general, similar to those reported in Caucasian populations, and there was some evidence for North-South gradient (Al-Araji and Mohammed, 2005). While its etiology(ies) is unknown, MS is clearly immune mediated disease influenced by genetic and environmental factors (Mikol et al., 2006; Dyment et al., 1997; Chabas et al., 2004; and Dyment et al., 2004). Infectious and inflammatory processes could play an important role in the pathogenesis of MS; C-reactive protein (CRP) is one of positive acute phase protein and it is synthesized in the liver and then exerted in the blood. CRP levels can be increased more than 10000 times as a response to an infection and represent direct and quantitative measure of acute and chronic inflammatory reaction (Vuksan-Ćusić et al., 2010). It has been shown to be associated with multiple sclerosis (Soilu-hanninen et al., 2005 and Assadi et al., 2010). Intrinsic factor (IF), which is also known as gastric intrinsic factor (GIF) is a glycoprotein produced by the parietal cells of the stomach. It is necessary for the absorption of vitamin B12 later on in the small intestine. In the humans, the gastric intrinsic factor protein is encoded by the GIF gene (Howard et al., 1996). Two types of intrinsic factor auto-antibodies exist (Carmel, 1992). Type 1 antibodies block the cobalamin (vitamin B12) binding site on the intrinsic factor molecule, preventing uptake of the vitamin. Type II antibodies block a different site of the intrinsic factor molecule that is involved in binding of the intrinsic factor –cobalamin-complex to ileal receptors. Both types of antibodies have the same pathological effect, i.e. preventing cobalamin resorption by ileal receptors (Chapel, et al., 2006). A vitamin B12 has very similar symptoms and is frequently misdiagnosed as MS both cause altered sensation, cognitive changes, muscular problems, and gastrointestinal upset (Stannard, 2010). Since the symptoms of B12 deficiency is overlap with that of MS; this study was designed to assess levels of high-sensitivity C-reactive protein (hs-CRP) and anti-intrinsic factor antibody (IgG) among MS patients in order to avoid confusion between MS and vitamin-B12 deficiency.

Patients and Methods

1-Patients:
Fifty-two patients with multiple sclerosis aged 14-54 years, Mean±S.E. (36.36±1.24), were enrolled from the medical city teaching hospital. MS patients were excluded if they had a known autoimmune or neurological disordered other than MS and 30 healthy blood donors aged 15-54 years, Mean (32.76±1.45) were taken as controls. They were drawn from spouses or friend of patients and from volunteers in the university of Baghdad, biology department; they were excluded if they had a known autoimmune or neurological disordered or history of MS in any family member.

2-Blood samples
Duration at time of serum collection was recorded; it was ranged between 1-18 years, Mean±S.E. (5.33±0.69). In most cases, patients were on interferon-beta (INF-β), some of them were psychiatric.

3-Immunological tests:
Tubes containing MS and control sera were labeled and carried out to measure high sensitivity C-reactive protein (hs-CRP) and anti-intrinsic factor (IgG) by using Enzyme-linked immune sorbent assay (ELISA). This was performed as described in
the leaflet of kits (DRG International, Inc; USA for hs-CRP and Immuchem, Belgium for Anti intrinsic factor).

4- Statistical analysis

The statistical analysis used included Student t-test and Pearson chi-square test ($\chi^2$). The Statistical Package for Social Science V.13 (SPSS) was used. A p-value <0.05 was considered statistically significant (Sorlie, 1995).

Results

A total of 52 MS patients were diagnosed of which 27 females (51.9%) and 25 males (48.1%) with a mean age 36.36±1.24 years and the mean duration being 5.33±0.69 years. According to the period of sample collection, a progressive increase in cases at third decade of age and trend towards more females was noted. The levels of hs-CRP and anti intrinsic factor IgG antibodies in MS patients and controls are reported in tables 1 and 2, we did not observe any differences in hs-CRP levels between MS patients and healthy controls (p>0.05), while there was a high significant increase in the level of anti-intrinsic factor IgG antibodies in MS patients’ sera compared with control group (p<0.05).

**Table 1: hs-CRP values in MS patients and healthy controls**

<table>
<thead>
<tr>
<th>Studied Group</th>
<th>N</th>
<th>Mean mg/L</th>
<th>S.d.</th>
<th>Student (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS Patients</td>
<td>52</td>
<td>1.294E-02</td>
<td>1.685E-03</td>
<td>0.25</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>4.300E-03</td>
<td>1.932E-03</td>
<td></td>
</tr>
</tbody>
</table>

*NS=non significant

**Table 2: Anti-intrinsic factor Ab (IgG) in sera of MS patients and controls**

<table>
<thead>
<tr>
<th>Studied Group</th>
<th>N</th>
<th>Mean U/ml</th>
<th>S.d.</th>
<th>Student (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS Patients</td>
<td>52</td>
<td>5.0770</td>
<td>5.4927</td>
<td>0.00 HS*</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>1.9877</td>
<td>0.6188</td>
<td></td>
</tr>
</tbody>
</table>

*HS=high significant

In the present study we report that serum hs-CRP and anti-intrinsic factor IgG antibodies levels in MS patients are not related to age of onset of the disease. Specifically, no significant differences were found in the levels of hs-CRP patients and anti-IF antibodies between those who experienced the onset of the first neurological symptoms prior to age 18 years (N=8), and the patients in whom the disease first manifested after age 18 years (N=44) p>0.05. As shown in tables 3 and 4.

**Table 3: Relationship between hs-CRP values and MS patients over & under 18 years**

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>N</th>
<th>Mean mg/L</th>
<th>S.d.</th>
<th>Student (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 18</td>
<td>44</td>
<td>1.295E-02</td>
<td>1.738E-03</td>
<td>0.391 NS*</td>
</tr>
<tr>
<td>Under 18</td>
<td>8</td>
<td>1.288E-02</td>
<td>1.458E-03</td>
<td></td>
</tr>
</tbody>
</table>

*NS=non significant

**Table 4: Relationship between anti-intrinsic factor IgG levels and MS patients over & under 18 years**

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>N</th>
<th>Mean U/L</th>
<th>S.d.</th>
<th>Student (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 18</td>
<td>44</td>
<td>5.2064</td>
<td>0.8308</td>
<td>0.662 NS*</td>
</tr>
<tr>
<td>Under 18</td>
<td>8</td>
<td>4.3655</td>
<td>2.0179</td>
<td></td>
</tr>
</tbody>
</table>

*NS=non significant
On the other hand; there was a statistically significant correlation between the levels of anti-intrinsic factor antibodies and the duration of disease (p<0.05); while there is no correlation between hs-CRP levels and the duration of disease was observed (p>0.05) as shown in table 5.

Table 5: Correlation between hs-CRP, anti-IF- IgG and the duration of the disease

<table>
<thead>
<tr>
<th>hs-CRP &amp; Duration</th>
<th>N</th>
<th>Correlation</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IF Abs &amp; Duration (years)</td>
<td>52</td>
<td>-0.167</td>
<td>0.236</td>
<td>NS</td>
</tr>
</tbody>
</table>

Discussion

From a total of 52 MS patients, 27 were females (51.9%) and 25 were males (48.1%) with a mean age 36.36 years. Within a given population, a progressive increase in cases at third decade of age and trend towards more females was noted. In line with the results of other studies, the overall prevalence of MS is approximately twice as high in women as in men. However, it is uncertain as to why more women than men contract the disease. Since the 1980s, an increasing number of cases of MS in children (under 18 years of age) have been recorded worldwide; and the initial symptoms have been seen as early as 13 months old, with diagnosis as young as two years of age (National Multiple Sclerosis Society, 2010). Most investigators consider inflammation central to the pathogenesis of multiple sclerosis (Giovannoni, 2001 and Link, 2001). Multiple components of the immune system, namely T-cells, B-cells, antibodies, interleukins and the innate immunity contribute to the tissue damage in MS (Meinl, 2010). However, to date the role of the important inflammatory mediator CRP has not been fully described. We did not observe any difference in hs-CRP between MS and controls. Our results are in line with the result of other studies showing that CRP values were similar in both MS patients and controls (Soiluhanninen et al., 2005; Meinl, 2010 and Fjeldstad, 2011). Actually, all MS cases have been received interferon-beta (IFN-β) in addition to another drugs, some of these patients were psychiatric; this may cause decrease of CRP levels. One study reported that after IFN-β injection, all inflammatory responses were attenuated at week 12 of therapy in comparison to those following the initial injection in a group of follow-up patients (Boylan et al., 2001). Other recent studies showed that the IFN-β treatment is associated with increased serum levels of hs-CRP in MS patients; in contrast when atorvastatin or simvastatin is added to IFN-β, hs-CRP serum levels decreased to the normal range indicating the anti-inflammatory action of these drugs in MS (Sellner et al., 2008; Devaraj et al., 2006 and Boyles, 2011). A recent study have shown the negative relationship dietary fiber ingestion and inflammatory markers like CRP (Lottenberg, 2010). However, a factor to be considered is the time course of raised systemic inflammatory markers, which occur as a result of an active MS lesion. The link between early relapse rates and disease progression has required follow-up studies lasting many years to show meaningful results. This would support the hypothesis that the effect of inflammation on disease progression is delayed (Giovannoni et al., 2001). Concerning the duration of the disease; no association was found with the mean levels of hs-CRP in MS patients. Just as other studies indicated; the mean levels of inflammatory markers from the six months of their study did not differ between patients who did or did not develop cerebral atrophy over the study period (Giovannoni et al., 2001). While anti-intrinsic factor antibody levels were statistically related to chronicity of illness, these finding suggest a specific association between the timing of onset of first neurological symptoms of MS and vitamin B12.
metabolism. This study revealed a significant elevation of anti-intrinsic factor IgG antibodies in MS patients compared to matched controls. As there is a strong evidence that MS is a disease caused by nutrient deficiency including vitamin B12; our result was concordant with the results of other studies which showed that frequency of biologically sever cobalamin(B12) deficiency in patients with MS was very low(Goodkin et al.,1994 and Van Rensburg et al.,2006). In contrast, one recent study showed that the percentage of anti-intrinsic factor IgG was 0% in MS patients (Banati et al.,2011). Like several previous studies; patients with MS had significantly lower serum vitamin B12 levels and they had significantly lower blood cell folate levels than neurological and normal controls (Schilling and Williams, 1995; Reynolds et al., 1992; Sandyk and Awerbuch, 1993 and Palm and Hallmans, 1982). Some metals are involved in important reactions in the body during the course of disease; copper is necessary for the metabolism of iron in the body and deficiency of copper may cause anemia due to defects in iron metabolism. It is also used with zinc, iron, and vitamin B in the synthesis of phospholipids which are used in myelin formation (Eliaeson et al., 2008). Many previous studies were indicated a significant low serum levels of copper and zinc in MS patients than in control subjects (Eliaeson et al., 2008 and Palm and Hallmans, 1982). On the other hand, prolonged iron deficiency anemia is associated with gastric and atrophy of glands producing intrinsic factor in the stomach ( Van Rensburg et al., 2006) or a condition, such as Crohn's disease, which affect the absorption of the vitamin B12 this in turn leads to inhibition of vitamin B12 uptake (Stannard ,2010). Anti-intrinsic factor-IgG antibody tests were able to detect the vitamin B12 deficiency. However, a number of available reports indicated that some people have normal blood levels of B12, but are unable to metabolize it. This is known as cobalamin G, which is hereditary (Tweet ,2011). Since vitamin B12 is required for the formation of myelin sheath and for immune mechanisms, we propose that its deficiency in MS patients is of critical pathogenic significance. So vitamin B12 deficiency should always be looked for in MSpatients.

References


Immunometric enzyme immunoassay for the quantitative determination of IgG class auto antibodies to intrinsic factor. (2005). instruction for use, Immuchem, Belgium.


Stannard, L. (2010). Comparison of Symptoms of B12 Deficiency & Multiple Sclerosis Nov.1, Merck Manuals Online Medical Library: Multiple Sclerosis (MS) and Related Disorders.


