Estimation of Serum Anti-Cyclic Citrullinated Peptide, Glutathione, Copper and Zinc in Patients with Multiple Sclerosis

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ABSTRACT
   This study aimed to determine the level of anti-cyclic citrullinated peptide (ACCP), glutathione (GSH), copper (Cu) and zinc (Zn) in sera of the patients with Multiple Sclerosis (MS) and healthy subjects to examine their possible role in this disease. Thirty patients in MS were included for evaluation of serum anti-cyclic citrullinated peptide (ACCP), glutathione (GSH), copper (Cu) and zinc (Zn) and they compared with thirty normal healthy subjects. Results revealed that the serum levels of GSH and Cu were highly significant lower in MS patients than in healthy control group $p < 0.005$. The mean serum levels of Zn for both groups were significantly decrease $p < 0.04$, but the mean serum levels of ACCP in both groups were no significantly decrease $p > 0.201$.

تقدير الأجسام المضادة للسترولين الحلقي والكلوتاثيون والنحاس والزنك
في أمصال المرضى المصابين بالتصلب المتعدد

الخلاصة

   هذه الدراسة تهدف لتحديد مستويات الأجسام المضادة للسترولين الحلقي والكلوتاثيون والنحاس والزنك في أمصال مرضى مصابين بالتصلب المتعدد والمنتشر في الجهاز العصبي والأشخاص الأصحاء لأختبار الدور الممكن لهذا المرض. تضمنت الدراسة ثلاثون مريض مصاب بالتصلب المتعدد لتقدير الأجسام المضادة للسترولين الحلقي والكلوتاثيون والنحاس والزنك وتمت مقارنتهم

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مع ثلاثين عينة من الأشخاص الأصحاء. أظهرت النتائج بأن هناك نقصان مؤثر عالي في تراكيز الكلوتاثيون والنحاس في أمصال المرضى مقارنة بالأشخاص الأصـــحاء($p> 0.005$) وهنــاك نقصان مؤثر في تركيز الزنك في أمصال المرضى مقارنة بالأشـــخاص الأصـــحاء($p> 0.04$) ولكن لم ينخفض تركيزالأجسام المضادة للسترولين الحلقي فـــي أمصـــال المرضـــى مقارنـــة بالأشخاص الأصحا($p > 0.201$) .

الكلمات المرشدة: التصلب المتعدد، الأجسام المضادة للسترولين الحلقي، الكلوتاثيون، النحـــاس، والزنك

INTRODUCTION

Multiple sclerosis (MS) is a progressive demyelinating process occurs as a result of the inflammation, considered as an autoimmune disease (1). Although different mechanisms may contribute to demyelination and neurodegeneration in multiple sclerosis, it recently became clear that mitochondrial injury and subsequent energy failure is a major factor driving tissue injury (2, 3, 4). The exact etiology of (MS) is still uncertain (5). Multiple sclerosis (MS) is characterized by a series of biochemical changes affecting neuronal functions (6), some of which are in common with other neurodegenerations such as Alzheimer's (7) and Parkinson's diseases (8). An in vivo biomarker could be a useful tool, enhancing our understanding of a possible, post-inflammatory disease process and perhaps serving as a surrogate marker to monitor disease progression in the later stage of MS (9). Anti-cyclic citrullinated peptide (anti- CCP) antibodies are antibodies against synthetic citrullinated peptides (10). Anti-CCP antibodies have been evaluated in a large number of patients with different autoimmune (11, 12, 13, 14) and infectious diseases (15, 16). Citrulline is an a typical amino acid formed by the deimination of arginine in certain proteins, including fibrin, vimentin, and filaggrin (17). Glutathion (GSH), a sulfahydryl (SH) – containing tripeptide, has several major physiological functions: it maintains SH groups of proteins in a reduced state, participates in the transport of amino acids detoxifies foreign compounds, enzymatically degenerates endogenous peroxides, form bioactive molecules and acts as a coenzyme in several enzymatic reactions (18). Zinc has an important role in inhibition of potentially destructive immune reactions against T lymphocytes and predisposing inflammatory responses of MS. It is also an antioxidant protecting cell membranes and myelin (19). Copper is needed for basal metabolic activities of bone, skin, and nervous system, and more importantly it is needed for enzyme reactions involved in the production of ATP, and transmission of impulses in nerves and muscles (20). The present study aimed to examine the levels of anti-CCP antibody, SH, Zn and Copper in sera of patients with MS.

MATERIALS AND METHODS

Selection and Clinical Evaluation of the Patients. This study was performed during the period from April 2011 to February 2012 and thirty MS patients age ranged between (20-60) years, mean ages (39.95 ± 1.1 years) were included in this study. They were assessed clinically at the out patient of Baghdad teaching hospital in medical city according to the McDonald criteria (20). The control group consisted of 30 healthy subjects, mean ages (42.81 ± 2.16 years), matched for ages and gender to the patients group. All selected subjects had no acute or chronic pathologies.

Preparation of Samples for the Serum assays. In both patients and controls, peripheral venous blood samples were collected from the antecubital vein into VACUETTE polypropylene tubes. After 25 minutes at room temperature, samples were centrifuged at $1890 \times g$ for 10 min to separate sera. The serum was separated from the whole blood after centrifugation and stored frozen until the assay.

Laboratory methods. Anti-CCP antibodies were measured using a commercial anti- CCP ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG, Lu¨ beck, Germany) according to the manufacturer's instructions. The GSH levels were estimated by the method described by Moron et al (21). Briefly 0.1 ml of serum was precipitated with 5% TCA and the precipitate was removed by centrifugation. To an aliquot of the supernatant 2ml of 5-5´-Dithiobis, 2-nitrobenzoic acid (DTNB) reagent was added to make the final volume three milliliter. Absorbance was read at 412 nm against a blank containing TCA instead of the sample. The amount of reduced glutathione was expressed as μmole / mg of protein. Zinc (Zn) and Copper (Cu) concentrations were determined by the flame atomic absorption spectrophotometry(20).

Statistical analysis

Statistical analysis was performed using SPSS (version 10). For comparing paired clinical data. In all cases $p \leq 0.05$ was taken as statistically significant.

RESULTS

A cohort of thirty patients with MS and thirty control groups were included in this study. Table 1 illustrated the mean±SD values of ages & BMI The mean age values were non significantly increase and mean values of Body mass index (BMI) were not significantly different in MS patients when compared to control group as shown in table (1).

A comparison of the serum ACCP, GSH, Zn and Cu levels in MS patients and control group were demonstrated in table 1.

Serum GSH and Cu levels were highly significantly decreased in MS patients compared to control group $p < 0.005$. The mean±SD values of Zn serum levels for both groups was significantly decreased $p < 0.04$, but the mean±SD values serum level of ACCP in both groups was not significantly decrease $p > 0.201$.

DISCUSSION

Since anti-CCP antibodies are gaining popularity as Rheumatoid Arthritis (RA) - specific serologic markers, it is important to investigate the possible mechanisms underlying this marker. Numerous studies have corroborated the high specificity of the anti-CCP assay for the diagnosis of (RA), and its association with erosive inflammatory joint disease has added to the prognostic value of anti-CCP antibodies. The major new finding in this study is that patients with MS has no significant difference in the level of serum ACCP as compared with control group, further large-scale studies are warranted. To our knowledge it's the first study that includes the measurement of ACCP level in sera of patients with MS disease. In the present study, GSH, Cu and Zn proved to be equally useful and specific to distinguish MS patients. Calabrese et al (22), found significant decrease in reduced glutathione and significant increases in oxidized glutathione and S-nitrosothiols in MS patients compared with control group, Choi et al (23), in their study of seventeen patients with SPMS (secondary progressive multiple sclerosis) and gender-matched healthy controls were measured GSH levels and found that The levels of GSH were lower for SPMS patients than for control. In this study there was difference in total serum Zn and Cu levels in patients with MS and control groups. Palm et al in their study on 50 MS patients showed that serum Zn and Cu levels were lower than their control group (20). In another study, Masoud & Fakharian (24), have stated that Zn and Cu levels in 35 MS patients were significantly decreased compared with control group which is compatible to our study.

In conclusion, the findings of this study and some other previous ones suppose that decreased serum GSH, Zn and Cu levels in MS patients may be an important indicator of the disease.

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Table (1): Mean±SD values of clinical characteristic in serum of patients with MS in comparison with control groups.

Parameters	Control n=30 Mean±SD	MS patients n=30 Mean±SD	P-value	Significant
Age(years)	39.95±1.10	42.81 ± 2.16	0.42	NS
BMI (Kg/m$_2$)	28.53 ± 0.56	27.64 ± 0.89	0.21	NS
ACCP (u/ml)	9.68 ± 0.98	9.62 ± 0.85	0.201	NS
GSH (μmole/L)	0.596 ± 0.101	0.268 ± 0.05	0.005	HS
Cu (μg/dl)	115.34 ± 24.32	81 ± 9.56	0.005	HS
Zn(μg/dl)	111.33 ± 11.65	96 ± 8.54	0.04	S