The Interplay between Inflammation and Metabolic Disturbances in Multiple Sclerosis

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Abstract: Background: Multiple sclerosis (MS) is a complex inflammatory, demyelinating and neurodegenerative disease with a heterogeneous pathology and clinical outcomes. Chronic inflammatory processes characterizing MS is claimed to be linked to systemic lipid metabolism and insulin resistance (IR). **Aim:** To assess the metabolic changes in MS patients and their relation to liver function. **Patient and method**: This case-control study included 30 individuals. They were divided into 2 equal sex and age matched subgroups; MS and control groups. For each group, lipid profile, glucose, insulin levels, HOMA-IR indices, aminotransferases, tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) were measured. Brain MRI and abdominal ultrasonography were done. **Results**: there was a significant elevation in glucose, insulin, HOMA-IR, TNF-α, CRP associated with dyslipidemia in MS group. However, there was no difference in aminotransferases levels between groups. Abdominal US revealed fatty liver in 66.7% of patient group vs 13.3% in control group. ***Conclusion:*** Inflammatory process and IR in MS patient is associated with non alcoholic fatty liver disease (NAFLD).

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1. Introduction

Multiple sclerosis (MS) is the most common cause of neurological disability worldwide. It commonly affects young and middle-aged people.(1)

It is an autoimmune disorder characterized by lesions in central nervous system (CNS) white matter, axonal degeneration, and cognitive impairment.(2)

The etiology of MS is not fully understood, but available evidence indicates that immune system may play an important role in its pathogenesis.(3)

Martins et al.(4) suggested that increased production of T-cell-derived cytokine as interleukine-6 (IL-6), IL- 12, IL-17 and tumor necrosis factor-α (TNF-α) may have a role in the pathogenesis of MS.

Cholesterol is an important component of intact myelin. Lipids, especially lipoproteins, are involved in the regulation of neural functions in the CNS through local mechanisms that are linked to systemic lipid metabolism.(5)

Moreover, high concentration of HDL present in CNS as a result of transport across the blood-brain barrier(6) has immunomodulatory and anti-oxidant effects on endothelial cells(7) and it has been shown to inhibit production of the pro-inflammatory cytokines IL-1β and TNF-α.(8)

Dalgas et al.(9) reported that triglyceride (TG) and cholesterol levels of MS patients were higher compared to healthy subjects.

Additionally, ***Weinstock-Guttman et al.***(10) have demonstrated that the lipid profile can adversely affect MS progression, particularly higher low-density lipoproteins (LDL) and total cholesterol (TC) and lower HDL levels that are associated with more inflammatory activity in MS patients.

In addition, dyslipidemia can potentiate inflammatory processes (11) and this is well established in conditions such atherosclerosis, cardiovascular disease, metabolic syndrome.(12)

In contrast, **Penesova *et al***.(13) found normal lipid profile and levels of inflammatory mediators in MS patients.

Moreover, MS may develop due to the neuronal imbalance in oxidants/antioxidants, with excess oxidants as reactive oxygen species (ROS)(14) and defective antioxidant as uric acid.(15)

Recently, it was demonstrated that there is increased insulin resistance (IR) prevalence and the association between IR and adiposity with disability in patients with MS. Additionally, IR was associated with chronic inflammatory process and oxidative stress in those patients(16).

Further, both systemic and hepatic IR with concomitant increased insulin secretion is associated with elevated liver enzymes and decreased hepatic insulin clearance.(17)

Interestingly, it was reported that patients with MS have also elevated liver enzymes(18). However,

Chan *et al.* [19] claimed that abnormal liver function in MS patients developed after receiving MS treatment.

Therefore, this study was designed to assess (i) Plasma lipid profile. (ii) The liver enzymes changes and their relation to plasma lipid profile, inflammatory mediator and IR in Egyptian MS patients.

2. Patients and methods

Thirty individual were included in this study. They were divided to 15 MS patients and 15 age- and sex-matched healthy persons. They were assessed clinically at the Neurology outpatient clinic, Zagazig University. The age range of the patients was 31-56 years, and that of normal control subjects 28–55 years

All selected subjects had no acute or chronic pathologies. It should be noted that none of the patients had received immunosuppressive or immune-modulatory therapy for at least three months prior to sample collection. The study was approved by the local Ethic Committee. Written informed consents were obtained.

- *Blood sampling*

Five ml of venous blood were collected under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 _ g for 15 min). The separated serum was stored at −20°C for subsequent assay of:

1. TC by enzymatic colorimetric method according to ***Dietschy et al***.[20] (Biovision, San Francisco. Cat: K623-100).
2. TG by enzymatic colorimetric method according to ***McGowan et al***[21] (Biovision, San Francisco**.** Cat:K952-400).
3. HDL by colorimetric method according to ***Assman et al***[22] (Biovision, San Francisco. Cat: K613-100.
4. LDL was calculated using the equation: LDL= Total cholesterol _ HDL _ TG/5. Provided that serum TG level is <400 mg/dL[23].
5. Glucose by enzymatic colorimetric method according to ***Carroll et al.***[24] (Sigma, Aldrich, Cat: MAK083**).**
6. Insulin by enzyme-linked immunosorbent assay (ELISA), according to **Temple,** [25] (Sigma, Aldrich, Cat: RAB0327).
7. The homeostasis model assessment-insulin resistance index (HOMA-IR). It was calculated using the equation: HOMA _ IR = insulin (μU/mL) x glucose (mg/dl) /405. The cutoff point to define insulin resistance corresponds to HOMA-IR=3.8. [26].
8. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels: by clorimetric method according to **Vassault,**[27] (Biovision, San Francisco , cat: K752-1 00 and K753-100 respectively).
9. C-reactive protein (CRP)Immuno-enzymometric assay kits according to **Kimberly**,[28] (Elabscience Biotechnology, USA. Cat: E-EL-H0043)
10. TNF –α by ELISA according to **Engelberts *et al.***[29] (Elabscience Biotechnology, USA. Cat:E-EL-H0109).

- *Body Mass Index (BMI) measurements:* The weight and height were measured. BMI was calculated as body weight (kg)/height^2 (m^2).
- *Magnetic resonance imaging(MRI) Analysis*: was conducted at the radiology department, Zagazig university hospital. MRI was done by 1.5 tesla super conducting magnet (achieva, Philips medical system. Contrast scan was done with Gadolinium DTPA at dose of 0.2/kg).

Technique: axial T1WI, T2WI and axial FLARE, Coronal T2WI and sagittal FLAIR. POST CONTAST T1.

- *Ultra sonography (US) scan**:*** Abdominal ultra sonography was done to all patients and control to assess the liver condition using GE Logiq P5 Ultrasound.

Statistical analysis:

The data obtained in the present study were expressed as mean ± SD for continuous variables and frequency (percentage) for categorical variables. Comparison of continuous variables means in two groups performed by Student's t test, while Fisher Exact test was used to compare categorical variables means. Correlations were calculated by the Pearson correlation model. P value less than 0.05 was considered significant. The statistical analysis was done by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA).

3. Results:

In the present study there was no significant difference between the two study groups regarding age and sex, however, BMI was significantly increased in patient group when compared with control subjects (p<0.001).

Moreover, there was a significant increase in serum glucose (p<0.01), insulin (p<0.001) and HOMA-IR (P<0.001) in MS group in comparison to control (Table 2).

Additionally, our results revealed a significant increase in serum TC, TG and LDL levels (P< 0.001), however, there was a significant decrease in HDL (p<0.001) in the same group (Table 2).

Regarding inflammatory mediators in MS patients, there was a significant increase in serum TNF-α and CRP (P<0.001) in comparison to control group (Table 2).

Table 1: Demographic and characteristics of study population

	MS	Control	*P* value
Age	46±8.3	43.5±9.5	0.45
Gender(females)	n=12(80%)	n=10(66.7%)	0.42
BMI(Kg/m^2)	29.03±1.9	25.8±1.58	<0.001*

* Significant vs. control

Table 2: Serum levels of metabolic parameters and inflammatory mediators

	MS	Control	*P* value
Glucose(mg/dl)	119±23.2	95.2±14.3	<0.01*
Insulin(µU/mL)	29.2±4.5	16.2±1.5	<0.001*
HOMA-IR	8.7±2.8	3.7±0.56	<0.001*
TC(mg/dl)	222±34.4	126±27.1	<0.001*
TG(mg/dl)	160.46±20.4	124.46±14.5	<0.001*
HDL(mg/dl)	48±4.1	57.86±7.5	<0.001*
LDL(mg/dl)	142.77±36	44.77±25.3	<0.001*
ALT(U/L)	27.86±5.8	23.46±4.1	0.07
AST(U/L)	32.26±4.5	28±6.7	0.054
CRP (mg/l)	2.4±0.5	0.61±0.1	<0.001*
TNF- α (Pg/ml)	1.66±0.5	0.53±0.1	<0.001*

* Significant vs. control

While, there was a non significant change in serum ALT (*P*=0.07) and AST (*P*=0.054) levels between groups (Table 2).

Furthermore, in MS group there was a significant positive correlation between TNF-α, HOMA-IR (*P*<0.001), ALT (*P*<0.05) and AST (P<0.05) (fig 2,3,4). In addition, there was a significant positive correlation between CRP, and the same parameters mentioned before (*P*< 0.001, *P*<0.01, *P*<0.01 respectively) (figs. 5,6,7).

Interestingly, from US scan results fatty liver was significantly more prevalent in MS group compared to control group (66.7% vs. 13.3%; *p*<0.05) (fig 1).

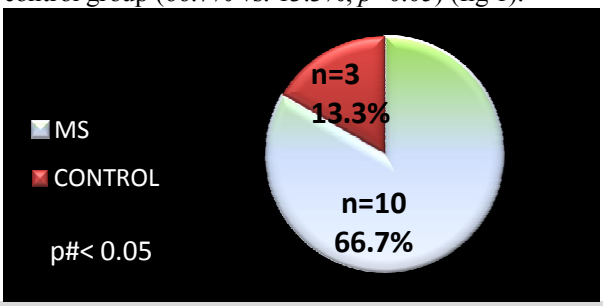


Fig (1): This chart represents the frequency of fatty liver according to US finding in both groups. (P# = significant Fisher Exact Test)

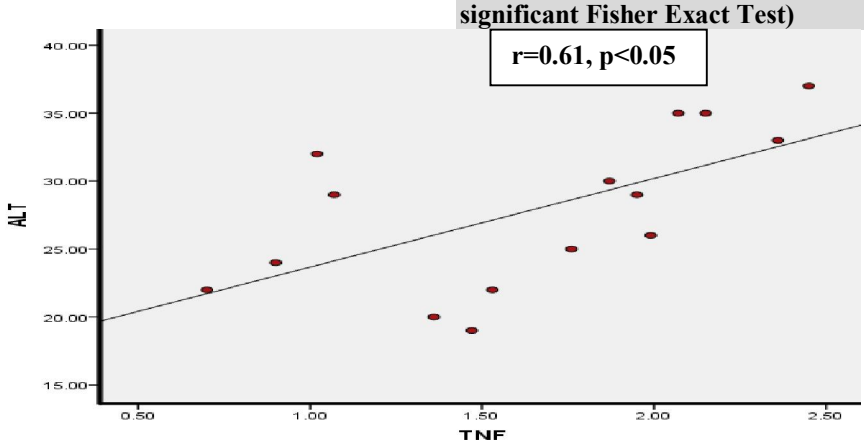


Fig (2): Correlation between TNF-α (pg/ml) and ALT(U/L) in MS group.

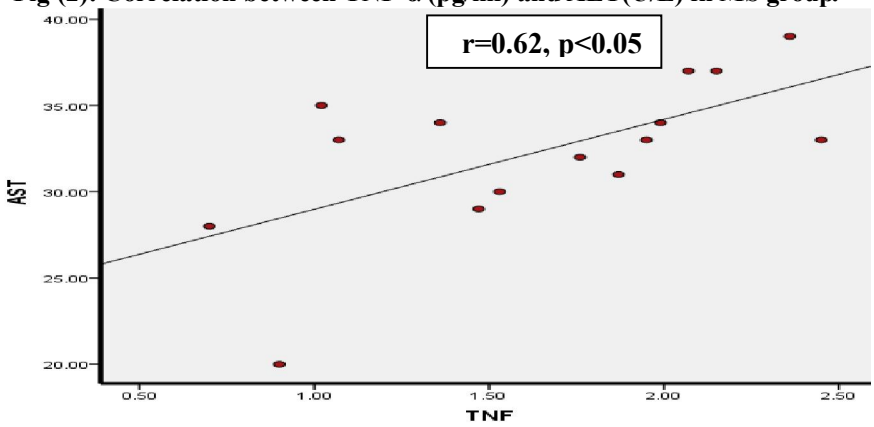


Fig (3): Correlation between TNF-α (pg/ml) and AST(U/L) in MS group.

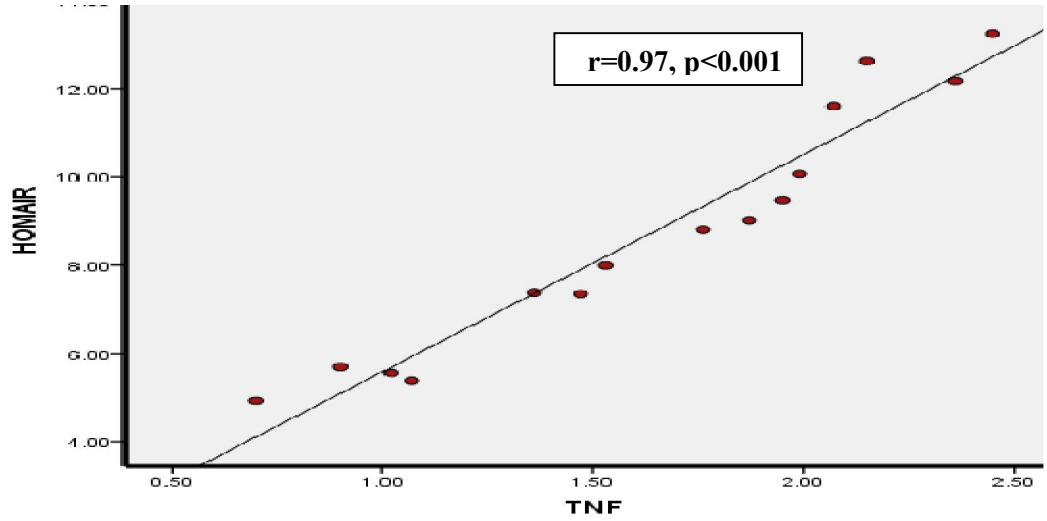


Fig (4): Correlation between TNF-α (pg/ml) and HOMA-IR in MS group.

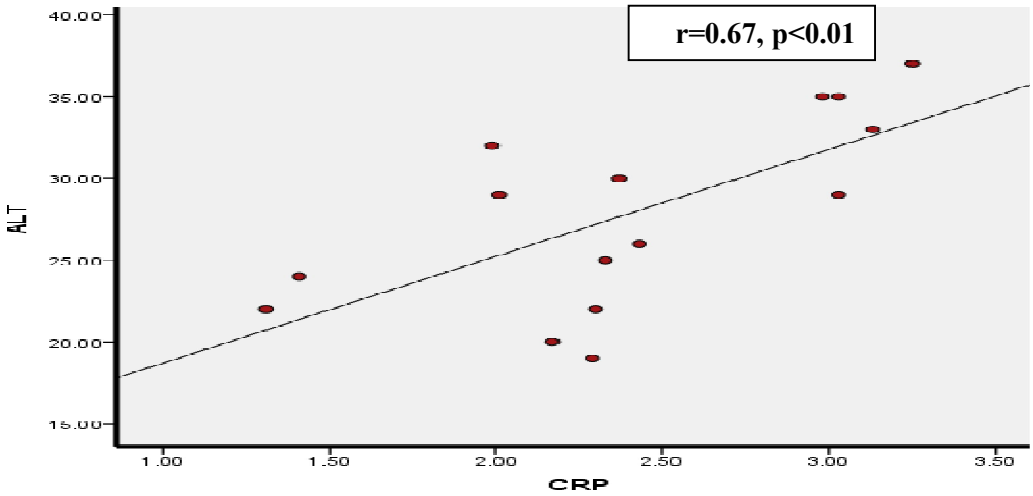


Fig (5): Correlation between CRP (mg/l) and ALT(U/L) in MS group.

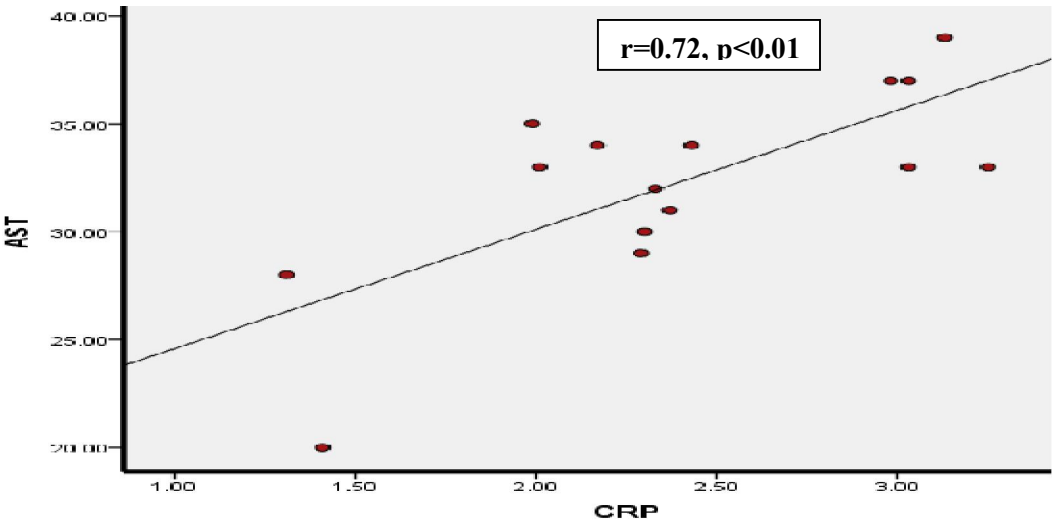


Fig (6): Correlation between CRP (mg/l) and AST(U/L) in MS group.

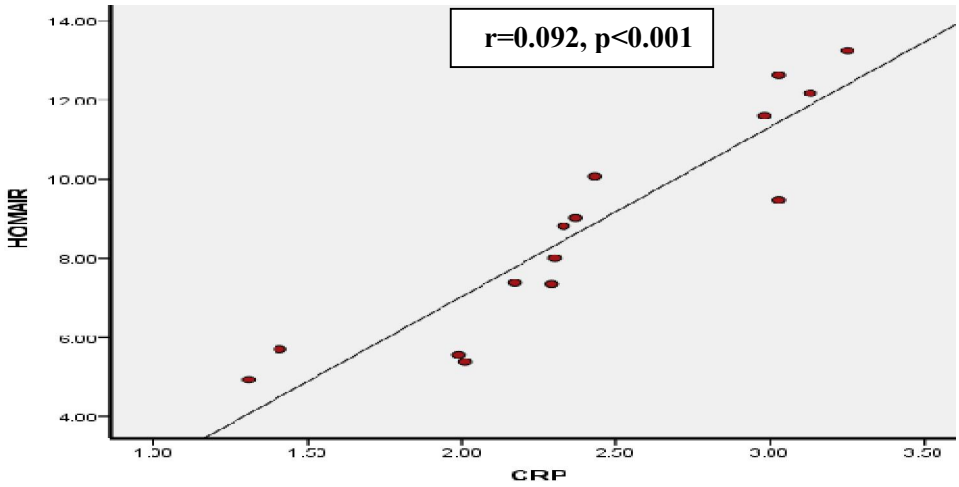
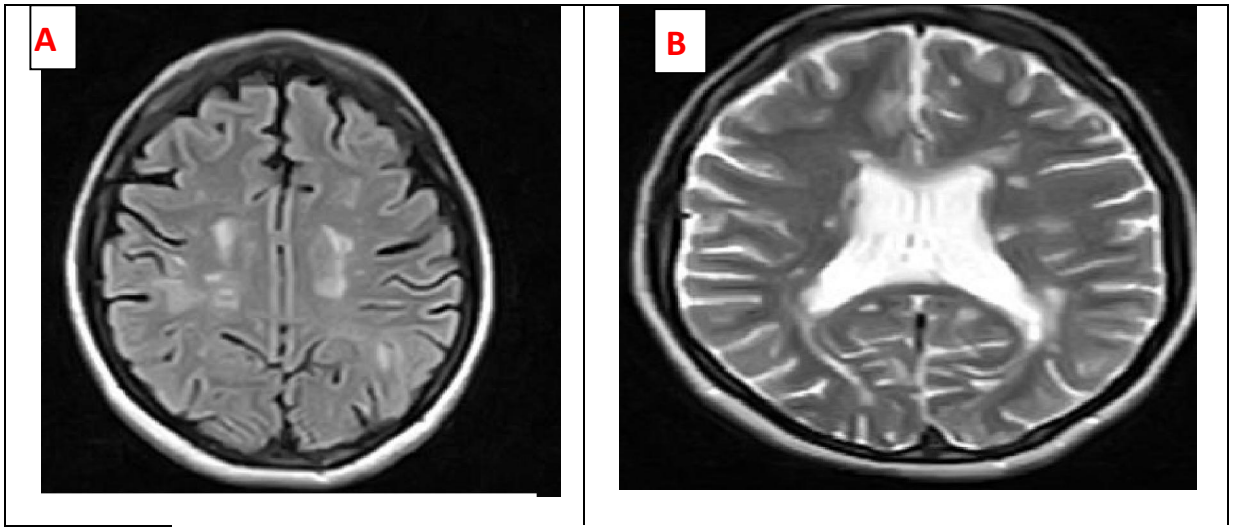
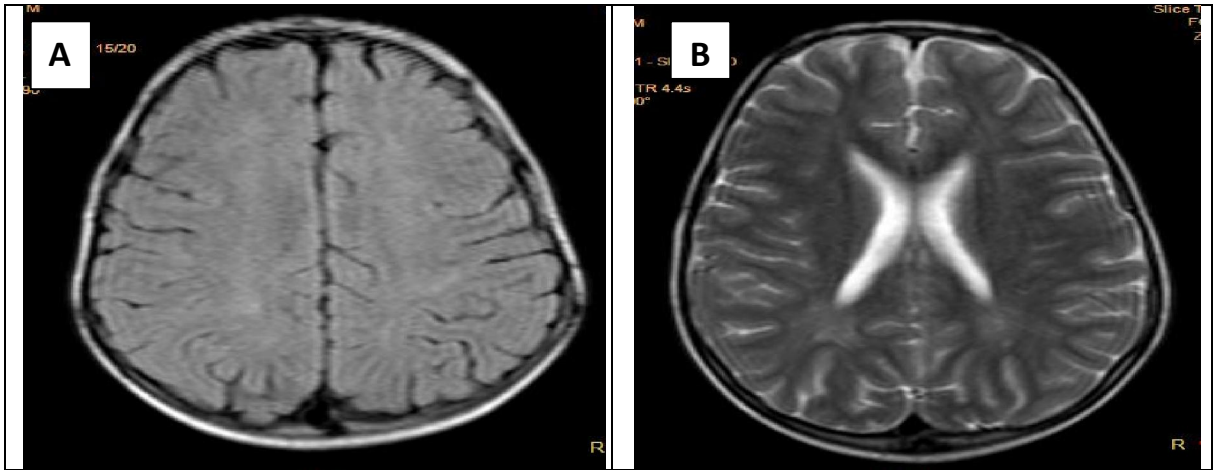


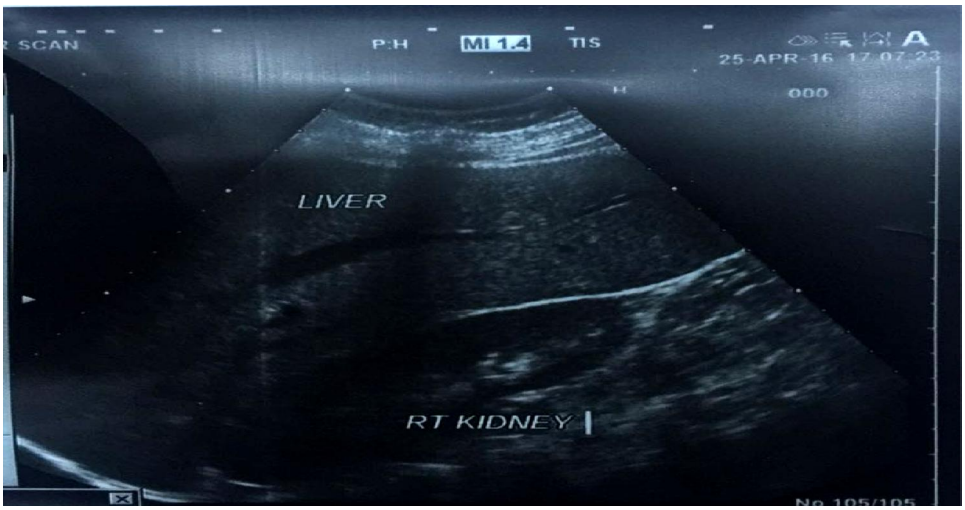
Fig (7): Correlation between CRP (mg/l) and HOMA-IR in MS group.



Picture i (A,B): Axial FLAIR and axial T2 at different level showing multiple abnormal peri-ventricular high signal intensities consistent with MS plaques.



Picture ii (A,B): Axial T2WI and axial FLAIR at different levels showing normal appearance of the white matter, no abnormal signal intensities.



Picture (iii): Abdominal ultra sonography: It reveals mild enlarged size, bright homogenous echogenic pattern liver with no focal masses or biliary dilatation indicating fatty liver.

4. Discussion

In the present study all patients were proved to be MS by MRI examination ***(picture i)*** & However, there were normal MRI imagines for all participants in control group ***(picture ii)***.

In addition, there was a significant increase in BMI, serum TC, TG, LDL, in MS group in comparison to control group. However, there was a significant reduction in serum HDL level in the same group.

Our results are in accordance with those of ***Monazamnezhad et al.***[30] ***& Bahrami et al***[31] who have shown that Sedentary lifestyle adopted by MS patients leads to increase body fat percentage and leptin hormone, an adipocytikine increased in obese persons due to development of leptin resistance[32]. It was also noticed that reducing BW, BMI, and body fat percentage in those patients can be achieved by caloric restriction[30].

Moreover, it was found that subjects with BMI exceeding 30 kg/m^2 at age 18 had more than a two-fold increased risk of developing MS compared with normal weight subjects. This result suggests a connective link between the obesity and the increasing MS incidence[33, 34].

In contrast, ***Formica et al***.[35] have reported that sedentary MS patients has decreased body weight which can be attributed to skeletal muscle atrophy.

Regarding lipid profile our results are in line with those of ***Weinstock-Guttman et al.***[36] who demonstrated that in MS increased TC, TG and LDL concentrations are associated with increased disability progression. Additionally, increased volume and number of contrast-enhancing lesion (CEL) in brain MRI were associated with lower HDL levels and higher TG and TC levels.

Interestingly, MS is characterized by derangement of brain metabolism caused by mitochondrial malfunctioning with excess ROS production[37], as a consequence there is excess demyelination indicated by an elevated cholesterol concentration in cerebrospinal fluid (CSF)[38].

Some authors reported that lipid metabolism abnormalities are not only limited to the myelin sheath, but also affect the plasma lipid profile of MS patients [39,38]and excess metabolites generated by transient or chronic dysfunction of brain metabolism will be found into the blood stream[40].

Moreover, in MS there are elevated CSF sorbitol, fructose concentrations, increases in plasma fatty acid levels and glutamic acid levels[41]which lead to increase in the nicotinamide adenine dinucleotide phosphate (NADPH) / NADP ratio. NADPH is used for acetate biosynthesis, the precursor of both cholesterol and TG[42].

Further, there was a significant increase in serum level of TNF-α and CRP in MS group in the present work in comparison to control group which is in line with the results of ***Emamgholipour et al.***[43] who demonstrated an elevation in circulating resistin, leptin, visfatin, TNF-α, IL-1β and CRP in MS patients.

This observation can be explained by the extravasation and recruitment of immune cells such as peripheral blood mononuclear cell (PBMC) and macrophages across the blood brain barrier activated vascular endothelium which is considered to be a critical step in MS pathogenesis and progression [43,44].

In addition, obesity as noticed in MS group is considered as a state of chronic low-grade inflammation which increases the production of

proinflammatory cytokines such as TNF-α, IL-6 and resistin[45].

Moreover, TNF-α stimulates the production of IL-1 which inhibits lipoprotein lipase activity leading to increase central fat deposition, plasma TG, LDL, while decrease HDL[46],adding additional explanation to the abnormal lipid profile observed in the patients group.

However, ***Penesova et al.***[13] found no significant difference in lipid profile and inflammatory mediators between patients and normal control.

Additionally, there was a significant increase in glucose, insulin levels and HOMA-IR indices in MS group in comparison to control group with significant positive correlation between HOMA-IR and inflammatory mediators (TNF- and CRP).

In accordance with our results ***Goldman et al.***[47]noticed that MS patients have hyperglycemia and at all oral glucose tolerance test (OGTT) time points they demonstrated statistically higher mean blood glucose levels.

In addition, ***Watson & Craft*** [2] reported that IR affects patients with MS and it may exacerbate manifestations of MS elevated inflammatory responses and cognitive impairments.

Interestingly, the thiazolidinediones which improve insulin sensitivity, reduce hyperinsulinemia, and exert anti-inflammatory actions have been proposed as potential therapeutic agents for both MS and Alzheimer's disease[2].

Our finding can be attributed to reduced mobility, sedentary lifestyle, as obesity is a major risk factor for IR[48]and repeated steroid administration which is common in MS patients [47].

Moreover, it was found that TNF-α enhances adipocyte lipolysis which increase phosphorylation of insulin receptor substrate-1(IRS1) which may block insulin signaling and lead to the occurrence of IR[49] which explains the positive correlation between HOMA-IR and both TNF-α and CRP in the present study.

Further, IL-1β impairs insulin signaling in macrophages and peripheral tissues, leading to reduce insulin sensitivity of β-cells and impair insulin secretion[50].

In addition, Resistin regulates the expression of proinflammatory cytokines including TNF-α, thus it plays a role in promoting IR [51].

However, ***Penesova et al.***[13] reported that decreased insulin sensitivity with in MS patients seems not to be related to chronic inflammation or physical inactivity.

Interestingly, in the present work abdominal US revealed fatty liver in 66.7% in MS groupvs13.3% in the control group (fig1) & picture (iii), despite the non significant elevation in serum ALT and AST in MS group in comparison to control group. But there was a significant correlation between liver enzymes and inflammatory mediators in the same group.

Our results are in line with ***Liao et al.***[52] who demonstrated that Liver function impairment usually develops after 6 months of interferon β-1a administration which is immunomodulation treatment for MS, further, after discontinuing interferon use the liver function was recovered.

In this study, no liver biopsy was performed due to the possible complications associated with this procedure[53]. However, ***Joseph et al*** [54] found an agreement between liver biopsy with histological examination and US scans in the diagnosis of fatty liver. Therefore, the diagnosis of non-alcoholic fatty liver disease (NAFLD) was based on US examination and on exclusion of alcohol abuse[55].

Our findings are in accordance with those of ***Hegazy et al.***[56] & ***Fracanzani et al.***[57] who demonstrated the presence of fatty liver with normal liver enzymes. Further, they reported that NAFLD tends to develop in people who are overweight, obese or have diabetes, high cholesterol or high TG.

NAFLD is one of the most common causes of chronic liver disease with increasing incidence in both adults and children, including a spectrum of liver disease ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis and even hepatocellular carcinoma[58].

IR evidenced in the present study may contribute to fatty liver development by reducing insulin-suppressing effect on hepatic glucose production which aggravates peripheral IR and contributes to hepatic lipogenesis[59] through increasing efflux of free fatty acids from adipose tissue[60] and inhibits their β-oxidation in liver promoting further hepatic lipid accumulation[61].

Moreover, ***Ajmal, et al.***[62] reported that patients with NAFLD have increased expression of genes that regulate inflammation leading to elevated CRP and TNF-α levels and they also noticed that their levels are positively correlated with the severity of fatty liver.

The production of biomarkers of inflammation and endothelial dysfunction by liver is up-regulated by IR and the metabolic syndrome. Furthermore, TNF-α is the major factor responsible for increased hepatic production of CRP, and other acute-phase proteins [63] which also explains the increased levels of these factors in MS patients.

Additionally, resistin which level is increased in MS patients[45], also have a role in pathogenesis of NAFLD, as increased resistin levels result in increased fatty acid synthesis, accumulation of TG, and reduced fatty acid oxidation in the liver via IR and inhibiting adiponectin action[64].

Moreover, it down regulates LDL receptors in primary hepatocytes, through increasing the

intracellular expression of the recently identified protease, proprotein convertase subtilisin/kexin type 9 (PCSK9), which enhances intracellular lysosomal degradation of LDL receptors[65,66], leading to a decrease in hepatic clearance of LDL with subsequent prolongation of its plasma half-life and, therefore, have steatogenic effects[67, 68].

In contrast, ***Rezapour-Firouzi et al.***[69] reported, elevated liver enzymes in MS that can be reduced by supplementation of diet with increased antioxidant capacity.

Regarding the prevalence of NAFLD demonstrated in control group in the present work, it seems to be in accordance with ***WeI et al.***[70] who found that one-fifth of the non-obese population has NAFLD. However, they have a low risk of developing steatohepatitis or fibrosis.

The discrepancy between our results and those of others may be attributed to genetic, age, sex, sample size, nutritional status differences and degree of metabolic disturbances of the participants in this study.

In conclusion, in MS patients, there is interplay between dyslipidemia, inflammation, IR and development of NAFLD which in turn aggravates the inflammatory condition and may have a role in progression of MS disease. Further, routine abdominal US scan for those patients is helpful for early diagnosis of liver diseases especially with normal aminotransferases during routine screening.

Recommendation: Further studies will be necessary to elucidate (i) The mechanisms by which adiposity, IR and inflammation could contribute to the progression and disability in patients with MS. (ii) If NAFLD is a cause or consequence of this disease.

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