Visual Evoked Potentials in Multiple Sclerosis Patients, Correlation with Glutathione Level as an Indicator of Oxidative Stress

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Abstract

Background: Multiple sclerosis implicit a high burden that is associated with reduced quality of life and a socioeconomic impact not only on the involved individual or family but on the whole health system. Glutathione is a thiol-containing molecule, as it functions as a main antioxidant Visually evoked potential refers to electrical potentials, initiated by transitory visual stimuli

Objective: the current study aim is to explore the correlation between glutathione level as a protective (antioxidant) and visual evoked potentials as a marker of early demyelination in MS patients. Patients and methods: This was a comparative cross-sectional study that was conducted in Ghazi Al Hariri Hospital from the first of November 2021 to the end of January 2022. Fifty patients diagnosed with multiple sclerosis were enrolled in the study and compared to 50 apparently healthy controls. For both groups visual evoked potentials were examined and glutathione level was measured using ELISA. All data were refined and analysis was done using SPSS version 20. Independent t test was used to explore the difference between the two groups. Pearson Correlation was calculated to investigate the type of the relation between glutathione levels and VEPS results in MS cases. In all statistical analysis a p value ≤0.05 is considered significant. Results: The average age of MS cases was 33.52±8.01 years. The mean disease duration was 4.06±3.05 years. The study showed a significant difference between MS cases and controls.MS cases had longer latency periods, lower amplitude, high IO differences, and lower glutathione levels. There is a negative correlation between the latency period and glutathione levels. Pearson correlation coefficient (r) was -0.418 (p- value = 0.002) for the left eye and r= -0.353 (p-value < 0.012) for the right eye. Another significant negative correlation was reported between disease duration and glutathione levels (r = -0.339; p value=0.016). Conclusions and recommendations: The relatively cheap, non-invasive procedure of VEPs helps in detecting the demyelination process, in association with glutathione levels that demonstrate the presence of oxidative stress, which highlights a promising therapeutic supplement for MS cases.

Keywords: Glutathione; GSH; MS; VEPS; evoked potentials.

1. Introduction

Multiple sclerosis (MS) is an incapacitating expensive chronic disease that is prevalent among working-age individuals (20-60 years) [1]. which implicit a high burden on work productivity that is associated with reduced quality of life and a very high socioeconomic impact not only on the involved individual or family but on the whole health system. In USA The estimated total economic burden was $85.4 billion while the total annual cost per patient ranged between 463$ and 58,616$ in low or middle-income countries [2, 3].

MS leads to a massive economic burden on healthcare systems and societies, knowing the fact that the Iraqi health system is barely recovering after series of unfortunate wars and displacements.

Glutathione is a thiol-containing molecule, as it functions as a redox buffer, main antioxidant, and enzyme cofactor against oxidative stress. In the brain, dysfunction of glutathione synthesis leading to glutathione depletion worsens the oxidative stress process [4]. Clinical studies revealed lower brain and plasma glutathione levels and reduced expression of inhibitor glutathione peroxidase in MS patients in comparison with healthy controls [5-7] which indicate the role of the oxidative stress in the pathogenesis of MS.

The term visually evoked potential (VEP) it refers to electrical potentials, initiated by transitory visual stimuli, a 50' checkerboard pattern is used, the evoked response is recorded using surface recording electrodes over the occipital lobe [8] The VEP magnitude (“amplitude”) and timing (“latency”) are affected by pathological changes [9].

Extension of VEP latency is considered as an indicator of demyelination; and the degree of demyelination within the visual pathway correlates with the delay [10].

The economic cost of MS is largely driven by indirect costs that are linked to early, and very high, unemployment rates, as it is considered a chronic disability. The costs increase dramatically with more severe incapacity, in particular when patients lose their upper limb function and independence [11].

The wide variation in causation theories, the fact that there's no specific cure for multiple sclerosis, at least no proven cure currently is endorsed. Though, treatments can help speed recovery from attacks, even modify the course of the disease and hopefully manage the symptoms in a better way, the cost and the hope for anything that can open a new opportunity to ease symptoms or halt progression for patients is definitely needed and thus the current study aim is to explore the correlation between glutathione level as a protective (antioxidant) and visual evoked potentials as a marker of

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early demyelination in MS patients.

2. Patients and Methods

This was a comparative cross-sectional study that was conducted in Ghazi Al Hariri Hospital from the first of November 2021 to the end of January 2022. Fifty patients diagnosed with multiple sclerosis and referred from the MS clinic during the study period were enrolled in the study and compared to 50 apparently healthy individuals (controls) with no MS or any differential diagnosis of MS disease like Behçet's disease; vasculitis; chronic meningitis, Sjögren's syndrome, antiphospholipid or anticardiolipin antibody syndromes etc.

Patients and controls were interviewed in the Nerve conduction velocity studies and Electromyogram clinics within the hospital premises and for both groups visual evoked potentials were examined and glutathione levels were measured

To measure the glutathione level, blood samples were withdrawn from each participant (3ml), centrifuged and kept in freezer (-20°C) until the time of analysis of glutathione level in the Central Teaching Laboratories of medical city as it is the main reference lab in Baghdad/Iraq. The Human glutathione enzyme-linked immune sorbent assay kit was provided by the researcher. The kit is a sandwich enzyme immunoassay for in vitro quantitively measurement of glutathione in human serum, plasma, tissue homogenates and other biological fluids. In the current study serum was used. Unopened kits were stored and kept according to the labels on vials. serum would be added to the wells, which are pre-coated with glutathione monoclonal antibody and then incubate, after that the anti-glutathione antibody labeled with biotin to unite with streptavidin-HRP is added, which forms immune complex. Adding substrates as the solution would turn blue and change into yellow due to the effect of acid. The shades of solution and the concentration of Human glutathione are positively correlated. Concentrations were provided by the lab directly.

To measure VEP, the patient is asked to sit comfortably in front of the screen. The stimulus; a patterned checkerboard shape stimuli was transmitted through a video monitor and producing checks that alternate from black to white and vice versa, was presented at 70 cm away from the patient. Electrodes are glued and wires were connected. Recording electrodes are placed on the scalp, additional electrodes are placed lateral to the midline occipital electrode. If the patient was un able to keep his/her eyes on the stimulus, then a short break was attended. The eye not being tested was patched. Usually, the procedure takes about 45 minutes.

All data were refined and analysis was done using SPSS (Statistical Package for the Social Sciences- version 20). Independent t test was used to explore the difference between MS cases and controls in regard of age, VEP latency, amplitude and intra ocular difference, and glutathione levels. Pearson Correlation was calculated to investigate the type of the relation between glutathione levels and VEPs results in MS cases. In all statistical analysis a p-value ≤0.05 is considered significant.

3. Results

The average age of MS cases was 33.52±8.01 years. The mean disease duration was 4.06±3.05 years Table (1) demonstrate the characteristic features of the studied sample. As for the healthy individuals (controls) the average age was 31.3±6.10 years. No significant difference in patients' age versus controls. However, the study showed a significant difference between MS cases and controls in regards to VEP results and glutathione level (p value <0.001). MS cases had longer latency periods, lower amplitude, higher IO differences, and lower glutathione levels

Table (1) comparison between MS cases and controls

Demographic variables	Groups		P value
	MS Cases (n=50)	Controls (n=50)	
	Mean ± SD	Mean ± SD	
Age in years	33.52±8.01	31.34±6.10	0.129
Disease duration	4.06±3.05		
VEPs			
RT P100latency (msec)	183.88±51.6	94.52±4.5	<0.001
LT P100latency (msec)	186.72±42.0	94.56±5.6	<0.001
RT amplitude (mv)	-1.22±0.78	6.09±0.90	<0.001
LT amplitude (mv)	-1.29±0.91	6.04±0.96	<0.001
IOD	42.06±29.9	4.72±3.23	<0.001
Glutathione level	3.97±1.81	14.36±6.69	<0.001

The current study showed a negative correlation between P100 latency period of the right and left eyes and glutathione levels. The longer the latency of P100, the lower glutathione levels. Table (2) shows the significant correlation between glutathione levels and multiple variables. Pearson correlation coefficient (r) was -0.418 (p value = 0.002) for the left eye and r= -0.353 (p value < 0.012) for the right eye.

Another significant negative correlation was reported between disease duration and glutathione levels (r = -0.339; p value=0.016), the longer the disease duration the lower glutathione levels reported.

Table (3.6) Correlation between glutathione levels and study variables

Variables		Glutathione	
		MS Cases	Controls
Age in years	Pearson Correlation	0.166	0.127
	P value	0.248	0.381
Lt. P100latency	Pearson Correlation	-0.418**	-0.120
	P value	0.002	0.405

Rt. P100latency	Pearson Correlation	-0.353*	-0.137
	P value	0.012	0.343
Lt. amplitude	Pearson Correlation	-0.016	-0.215
	P value	0.910	0.134
Rt. amplitude	Pearson Correlation	0.135	-0.146
	P value	0.351	0.310
IOD	Pearson Correlation	0.154	-0.004
	P value	0.285	0.975
Disease duration	Pearson Correlation	-0.339*	
	P value	0.016	
**Correlation is significant at the 0.01 level (2-tailed).			

4. Discussion

MS involves the working age individuals thus it is considered the most important disabling disease in adults [12]. As our current study revealed a mean age of MS cases was 33.52±8.01 years agreeing with results published by Singhal A et al, the mean age was 33.3±9.2 years [13] and Al Hussain H et al in Saudi Arabia where the average age was 32.07 years[14] in addition to several published literature from Iraq [15] and other countries [16] While the mean age was less than that reported by Younis A and Yahia A in Ninva -Iraq which was 39.5 years and also lower than that reported by Zaidi N et al mean age of 43.07 ± 13.88 years and in Lebanon by Zeineddine M et al who stated a mean age of 41.8 years [17] and likewise lower than the average age reported by Mellinger S et al from Argentina (42.1) years [18]. This discrepancy can be related to sampling techniques. Meanwhile, our current result was higher than that reported in a meta-analysis by Heydarpour P et al involved literature from Middle east and North African countries and revealed an overall mean age of 28.54 years for MS patients [19]. and also higher than outcome issued by Smagina IV et al study in Altai region of Russia where the average age of MS cases was 28.5±9.9 years [20] A difference might be inherent to the presence of other risk factors of having multiple sclerosis like genetic susceptibility, family histories, infectious mononucleosis, white race, temperate climates, vitamin D levels, other autoimmune disorders which is more prevalent in some countries [21-23].

The mean disease duration was 4.06±3.05 years comparable to result of a study by Singhal A et al, the duration was 5.98±4.95 years [13]. But shorter to that reported in Al hussain H et al research where the mean duration of the disease was 7.06 ± 4.7 years [14]. This can be related to better availability of care and treatments which influence patients' influx to the main hospitals.

The study showed a significant difference between MS cases and controls in regards to VEP results. MS cases had longer latency periods, lower amplitude, higher intraocular differences, which agrees with the results reported by Calugaru L et al where P100 wave were tested in patients with MS and compared to healthy subjects. The authors stated a significant difference between the two groups.[24] Our result is also in alignment with a study by Alshowaeir D et al who found that VEP latency in the MS cohort was significantly delayed compared to controls. [25].

A study by van der Weijden C et al and Alshowaeir, D et al, both studies confirmed a significant concordance and association between VEPs delay and prolonged latency period with the extent of optic lesion or lesion in posterior visual pathway optic lesions with VEPs delay [25, 26]. Thus, it was reported that VEPs had the advantage in detecting damage to the optic nerves more than MRI; although MRI have some superiority above VEPs, but to date, MRI has failed to provide outcome that are adequately sensitive and specific to remyelination process for example[27]. and in patients with preceding history of optic neuritis, would present with more delayed or even absent potentials than it could not be seen as an optic never lesion in MRI (Barton JL, Garber JY, Klistorner A, Barnett MH. 2019) VEPs have a proven role in evaluating demyelination along the optic nerve, as a functionally eloquent CNS region. Moreover, VEPs testing can be used to forecast the degree of recovery after optic neuritis and capture disabling effects of clinical and subclinical demyelination events in the afferent visual pathway[28, 29].

The current study showed a significant difference in glutathione levels among MS cases compared to controls agreeing with outcome of a study by Choi et al. [30] Patients with MS had substantially lower glutathione concentrations than controls[30]. The current study investigated this significant difference further and showed a negative correlation between latency period and glutathione levels. The longer the delay in VEPS indicates a demyelination process that is ongoing, as the lower glutathione recorded indicates more prominent involvement of oxidative stress contributing in the neurodegeneration likewise recent studies concluded that glutathione depletion introduces or is usually occurring before neurodegeneration and that is why neuronal glutathione depletion is considered the chief cause of neurodegenerative disease [31]. In augmentation the study also reported a significant negative correlation between disease duration and glutathione levels, the longer the disease duration the lower glutathione levels reported. This can be explained by the fact that as the disease progress with more relapses and continuous demyelination caused by active oxidative stress demonstrated by lowered glutathione levels, which goes in line with a study by Choi I et al where the authors concluded that patients with MS labeled to have worsening clinical status had significantly greater declines in glutathione concentrations than those with stable clinical status [32]. (Choi IY, Lee P, Hughes AJ, Denney DR, Lynch SG;2017) So the longer the duration, the more the demyelination, the lower the glutathione levels.

5. Conclusions and Recommendations

MS is a common disabling chronic disease, the relatively cheap, non-invasive procedure of VEPs helps in detecting the demyelination process, in association with glutathione levels that demonstrate the presence of oxidative stress, which could highlight a promising therapeutic supplement for MS cases.

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