

# Serum redox status, cytokines and vitamin D levels of Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder in Sri Lanka: A comparative pilot study

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## ABSTRACT

Multiple sclerosis (MS) and Neuromyelitis Optica Spectrum Disorder (NMOSD) are autoimmune demyelinating diseases of the central nervous system (CNS), with distinct pathophysiological significance. Understanding the cellular and immunological status may provide insight into the differential pathophysiology of these conditions and may improve the accurate diagnosis and management. This pilot study compares MS and NMOSD in Sri Lankan patients in terms of redox status, cytokines and serum vitamin D levels.

A total of 71 participants; 22 MS patients, 19 NMOSD patients, 15 disease controls (OND) and 15 healthy controls (HC) were recruited to compare serum oxidative parameters (OP); Nitric oxide (NOx) & Lactate dehydrogenase (LDH), antioxidant parameters (AP); Total antioxidant status (TAS) & Catalase (CAT), cytokines (Th1; INF $\gamma$  and Th2; IL-10) and vitamin D levels.

Serum levels of NOx ( $p=0.001$ ) differed significantly between the study groups and the MS patients exhibited significantly higher levels compared to NMOSD patients ( $P=0.002$ ). Serum LDH levels were comparable between both MS and NMOSD patients ( $P=0.07$ ). However, serum TASs significantly differed among the study groups ( $P=0.001$ ) and MS patients had near significantly lower levels compared to that of NMOSD patients ( $P=0.052$ ). Catalase activity levels did not show a significant difference among the groups ( $P=0.07$ ). The levels of IL-10 cytokines of MS patients were comparable to that of NMOSD patients ( $P=0.07$ ), however, it was significantly greater than OND ( $P=0.020$ ) and HC ( $P=0.032$ ) groups. The mean value of IFN- $\gamma$  in MS patients was 0.87pg/ml ( $\pm 0.145$ ) while IFN- $\gamma$  levels of other groups were less than the detection level of the standard ELISA kit. Vitamin D levels of MS and NMOSD were very similar ( $P=1.000$ ) and showed a resemblance to HC.

We conclude that oxidative stress and Th1 cytokines contribute more towards MS than NMOSD and that Vitamin D does not play a major role in disease pathogenesis in Sri Lankan patients.

*Keywords: Oxidative stress, Vitamin D, Multiple Sclerosis, Neuromyelitis Optica spectrum disorder, Sri Lanka, Demyelination*

## 1. INTRODUCTION

Multiple sclerosis (MS) and Neuromyelitis Optica Spectrum Disorder (NMOSD) are autoimmune demyelinating diseases with distinct pathological significance [1,2]. Multiple sclerosis (MS) is a heterogeneous disease resulting from demyelination of the myelin sheath of neurons which the pathogenesis is immune-mediated [3]. Although it had long been debated, NMOSD as a severe variant of MS, differential clinical manifestation characterized by optic neuritis and long lesion myelitis have established that the NMOSD as a different entity [1]. Further, NMOSD is differentially diagnosed by the presence of specific antibodies (IgG) against antigen aquaporin-4 (AQP4) and Myelin Oligodendrocyte Glycoprotein (anti-MOG) [4,5].

The global prevalence of MS has increased since 2013 and currently 2.8 million people are living with MS where the highest incidences have been reported the from European region [6]. According to 2020 data, the pooled global prevalence of NMOSD appears as 1 per 100,000 people with the highest prevalence in the African region [7]. In the long term, demyelinating diseases were inadequately studied in Asian regions due to the evidence of their higher prevalence in countries away from the equator [8]. However, recent findings suggest both MS and NMOSD incidences are increasing in Asian regions [6,7].

The natural history of MS and NMOSD in Sri Lanka is comparable to that of countries with high disease prevalence. Multiple sclerosis was first reported in Sri Lanka by Senanayake et al in 2001[ 9]. The current prevalence of MS is 9 per 100,000 population with around 2000 people living with the disease<sup>1</sup> and clinical diagnostics are based on the revised McDonald's criteria [10]. In an ongoing study published recently, 23% of 726 Sri Lankan patients presenting consecutively with all forms of Central Nervous System Inflammatory demyelinating diseases (CNS-IDDs) were seropositive for either AQP4-IgG (5%) or MOG-IgG (17.4%) with 25% of MOG-IgG seropositive, fulfilling the 2015 NMOSD diagnostic criteria [11]. The same cohort reports a 47% AQP4 seropositivity among 114 cases of NMOSDs and an estimated AQP4 seroprevalence of 0.25 per 100000 of the population. This infers a calculated prevalence of NMOSD of 0.54 per 100000 population [11]. Hence, it is inferred that both MS and NMOSD incidences are rising in Sri Lanka. Despite the differential diagnosis of NMOSD and MS in Sri Lanka, their entwined pathologies may complicate their prognosis and treatment [1]. Hence, it is a timely requirement to understand the status of these diseases for accurate prognosis & treatment and to predict the risk factors involved in the occurrence of MS and NMOSD among the Asian population.

Serum biomarkers offer an effective and economical option for the initial evaluation of the diseases and their progression and therapeutic decisions [12]. Redox markers [13] and cytokines [14] provide an excellent choice for understanding the heterogenic nature of neuroinflammatory diseases. Oxidative stress plays a major role in mediating the inflammation leading to demyelination and axonal of neurons [13,15]. Further, the disruption in the cellular antioxidant defence system increases the sensitivity of CNS to reactive oxygen species (ROS) [13]. A comparative analysis has exhibited high levels of oxidative stress markers and lower levels of antioxidants in MS and NMOSD patients in comparison with healthy controls [16]. This indicates redox markers may provide insight into the differential pathophysiological processes involved in MS and NMOSD.

Cytokines are soluble mediators of the immune system which directly involved in the onset and progression of both MS and NMOSD [17, 18]. A strong correlation has been observed between proinflammatory cytokine; interleukin-17 (IL-17), IL-22, IL-1, IL-12, tumor necrosis

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<sup>1</sup> <https://www.atlasofms.org/map/global/epidemiology/number-of-people-with-ms>

factor- $\alpha$ , (TNF  $\alpha$ ) and interferon- $\gamma$  (IFN $\gamma$ ) with the MS while levels of anti-inflammatory cytokines such as IL-4 and IL-10 were reduced [17]. A parallel study has observed a significant elevation of serum IL-6 levels in NMOSD patients [19]. Though, several studies have established correlations of cytokines with MS and NMOSD, a very few studies have conducted a comparative analysis. Hence, the present study envisages comparative analysis of cytokine levels [pro (IFN $\gamma$ ) and anti-inflammatory (IL-10)] in serum of both MS and NMOSD patients of Sri Lanka.

Vitamin D levels have been long recognized as an environmental risk factor in MS which relates with the higher prevalence in countries away from the equator and known to correlate with high relapse rates [20]. This further relates with the higher prevalence in countries away from the equator where availability of sunlight is lesser compared to tropical countries [20]. However recent studies have reported that it may not always be the same in the context of tropical and subtropical countries [21]. Identification of the prevailing serum vitamin D of both MS and NMOSD patients would help to understand the risk of developing MS or NMOSD in Sri Lanka.

Collectively, the present study sought to evaluate serum levels of oxidative stress markers, antioxidant parameters, cytokine and vitamin D of MS and NMOSD to obtain an extensive understanding of these diseases. Outcome of this study would also provide an insight into disease specific pathological mechanisms of neurodegeneration and facilitate in determining antioxidant therapy or immunotherapy as treatment strategies. Further, extensive and differential of these two distinct diseases entities would facilitate to understand the etiologies of MS and NMOSD that would explain the rise of these conditions in countries like Sri Lanka.

## **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

### **2.1 Ethical approval**

Ethical approvals for patient recruitment and sample collection were obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo (Protocol No. EC-18-039) and Ethical Review Committee, The National Hospital of Sri Lanka (Protocol-AAJ/ETH/COM/2018).

### **2.2 Study population**

This pilot study included randomly selected voluntary participants between the ages of 18-65 with informed written consent. The MS and NMOSD patients were recruited at the Multiple sclerosis and related disorders clinic, National Hospital of Sri Lanka who were diagnosed by a consultant neurologist. The diagnosis of MS was made based on 2017 revised McDonald criteria [22] and confirmed to be seronegative for both AQP4-IgG and MOG-IgG. NMOSD patients were seropositive for either AQP4-IgG or MOG-IgG and fulfilled the 2015 diagnostic criteria for NMOSD. Seropositivity was confirmed by cell-based assays carried out at Mayo Clinic USA as part of an ongoing collaboration with Sri Lanka. At the time of recruitment, all patients with MS and NMOSD were stable on immunotherapy without any acute exacerbation. Patients with autoimmune disorders, malignancies and chronic inflammatory conditions were excluded from the study.

### **2.3 Data and sample collection**

The socio-demographic data; age, gender, age at onset of symptoms, disease course, disease duration (RRMS in MS; ON/TM/Both in NMOSD; diagnosis in Disease controls), antibody status (AQP4/MOG) in NMOSD, EDSS score in MS were collected using an interviewer administrated questionnaire. The disease duration was calculated by subtracting the age at onset from the current age (Disease duration=current age – age at onset). The progression index which is a measure of accumulated disability over time was calculated by dividing the EDSS score by disease duration (Progression index=EDSS score/disease duration). From 3ml of venous blood collected by trained phlebotomists, serum was separated and aliquots were stored at -20°C until the assays were performed.

## **2.4 Biochemical analysis of serum oxidative and antioxidant parameters**

Analysis of serum Nitric Oxide (NOx) levels was done using the Griess reaction as per the procedure indicated in [23] with slight modification. The amounts of Lactate dehydrogenase (LDH) in serum were assessed using Gernon LDH kit (RAL Technica Para el Laboratorio, S.A, Spain). Total Antioxidant Status (TAS) in serum samples was determined as per Erel, 2004 [24]. The activity of the catalase enzyme was analysed by Hadwan and Abed, 2015 [25].

## **2.5 Cytokine analysis**

Serum levels of Th1 cytokine (IFN $\gamma$ ) and Th2 cytokine (IL-10) were assayed using human sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits as per manufacturer instructions (Becton & Dickinson OptEIA™, USA). Briefly, a standard 96-well microtiter plate (Immulon™2 HB, High Binding, USA) was coated with capture antibody and incubated at 4°C overnight for binding. The non-specific binding was blocked by adding assay diluent (PBS in 10% FBS) after washing the plate with wash buffer. The plate was then incubated at room temperature (RT) for 1 h. Serum samples, controls (only the assay diluent) and standards (diluted in assay diluent) were dispensed into respective wells and after 2 h of incubation, the detection antibody with the biotin-streptavidin enzyme conjugate was added. After 1 h, the substrate solution Tetramethyl Benzidine (TMB) was dispensed to the well and the plate was sealed. Then the plate was incubated at RT in the dark. The stop solution (2N H<sub>2</sub>SO<sub>4</sub>) was added, and the optical density (OD) of each well was read at 450 nm using a microplate reader (LisaScan II, Erba Mannheim, Germany). The concentration of IFN $\gamma$  and IL-10 in each serum sample was calculated using standard curves plotted for IFN $\gamma$  and IL-10 cytokines.

## **2.6 Serum vitamin D level analysis**

Vitamin D assay was performed using a 25-Hydroxyvitamin D Total ELISA kit as per manufacturer protocol (Global Diagnostics B, Vlasmeer 5, 2400 Mol, Belgium). Accordingly, reagents were prepared and, 25 $\mu$ l of each Calibrator, Control and Sample were added into the appropriate wells. 75 $\mu$ l of Incubation Buffer was added to the wells and incubated for 1 hour at room temperature. The liquid was aspirated from each well and the plate was washed 3 times by dispensing 350 $\mu$ l of Wash Solution into each well and aspirating the content of each well. Then, 100 $\mu$ l of the working HRP conjugate solution was added to each well and incubated for 15min at room temperature. The liquid was aspirated from each well and the plate was washed 3 times by dispensing 350 $\mu$ l of Wash Solution into each well and aspirating the content of each well. Following this, 100 $\mu$ l of the Chromogenic solution was added into each well within 15min following the washing step and incubated for 15min at room temperature in the dark. Finally, 100  $\mu$ l of Stop Solution was added into each well and absorbance was read within 1 hour at 450nm against 630nm (LisaScan II, Erba Mannheim,

Germany). The serum vitamin D levels of samples were determined from the calibration curve constructed.

## 2.7 Data analyses

The results are expressed as mean  $\pm$  S.E.M (Standard Error Means). The statistical analyses were performed using SPSS version 20 (IBM, USA). Analysis of variance with Tukey's post hoc analysis was used to compare the variables between groups having parametric data. The Kruskal-Wallis test was performed to analyse different groups having non-parametric data, followed by Mann-Whitney U test. Groups of categorical data were analysed by Chi-square and correlations were assessed using the Pearson and Spearman tests. The significance level was set at  $p < 0.05$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Demographic data

The clinical and demographic data are summarized in Table 1. All MS patients were of the subtype relapsing-remitting multiple sclerosis (RRMS). In cases with an eventual diagnosis of NMOSD, 66.7% and 26.7% had either optic neuritis or transverse myelitis respectively as the initial presentation. Only 6.7% had simultaneous optic neuritis and transverse myelitis at presentation. Most patients were seropositive for MOG-IgG (80%) relative to AQP4-IgG (20%). Higher female predominance was seen in NMOSD patients than in MS patients ( $P=0.005$ ). The age ( $P=0.010$ ) and age of onset ( $P=0.001$ ) of MS patients were lower than that of NMOSD patients. Accordingly, MS patients had longer disease durations ( $P=0.053$ ). In MS patients the mean expanded disability status scale (EDSS) score was 1.80( $\pm 0.28$ ) and the mean progression index was 0.69 year ( $\pm 0.16$ ).

**Table 1. Demographic & clinical parameters of participants.**

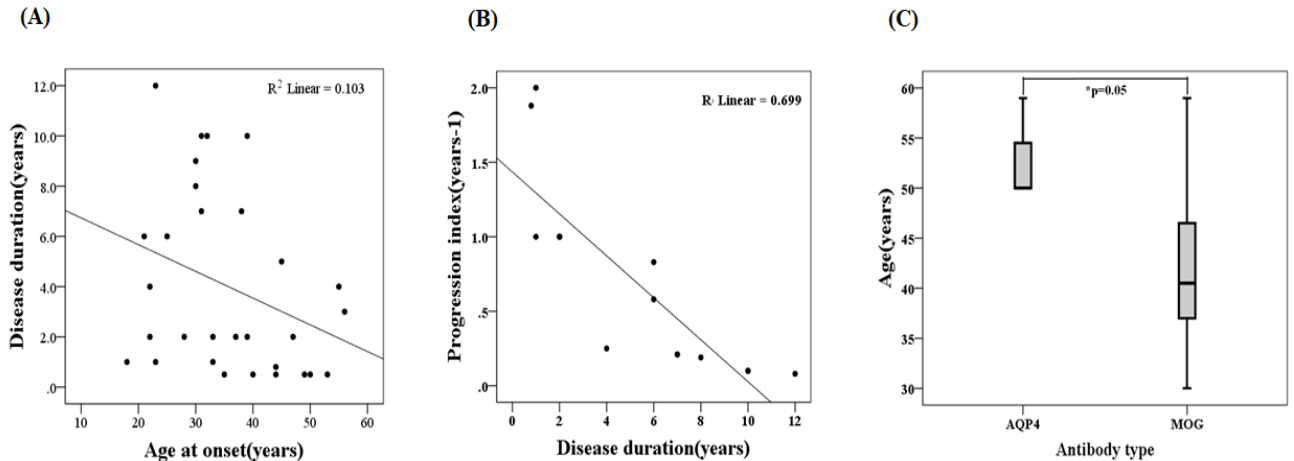
Sample groups	Test groups			Control groups	
	MS (n=22)	NMOSD (n=17)	P-value (MS/NMOSD)	OND (n=15)	HC (n=15)
<b>Gender [%]</b>					
male/female	50/50	17.6 / 82.4	<b>0.008*</b>	66.7 / 33.3	20 / 80
<b>Age [years]</b>	34.68 $\pm$ 2.04 <sup>†</sup>	44.65 $\pm$ 2.02 <sup>†</sup>		43.60 $\pm$ 3.49 <sup>†</sup>	32.13 $\pm$ 2.44 <sup>†</sup>
	(19-54) <sup>‡</sup>	(30-59) <sup>‡</sup>	<b>0.019*</b>	(21-62) <sup>‡</sup>	(25-57) <sup>‡</sup>
<b>Age at onset[years]</b>	29.36 $\pm$ 1.89 <sup>†</sup>	42.18 $\pm$ 2.14 <sup>†</sup>		42.60 $\pm$ 3.63 <sup>†</sup>	-

	(15-47) <sup>‡</sup>	(28-56) <sup>‡</sup>	<b>0.00007**</b>	(15-60) <sup>‡</sup>	
<b>Disease duration[years]</b>	5.4±0.91 <sup>†</sup> (0.80-15.0) <sup>‡</sup>	2.6±0.70 <sup>†</sup> (0.50-10) <sup>‡</sup>	<b>0.031*</b>	1.30±0.38 <sup>†</sup> (0.5-6.0) <sup>‡</sup>	-
<b>EDSS score</b>	1.81±0.20 <sup>†</sup> (1.00-5.00) <sup>‡</sup>	-	-	-	-
<b>Progression index[years<sup>-1</sup>]</b>	0.72±0.14 <sup>†</sup> (0.08-2.00) <sup>‡</sup>	-	-	-	-
<b>Disease course[%], ON/TM/Both</b>	-	64.7 / 29.4 / 5.9	-	-	-
<b>Antibody[%], AQP4/MOG</b>	-	23.5 / 76.5	-	-	-

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Data are presented as mean ± standard error of mean where applicable. Statistical analysis was performed using Independent-samples T test for continuous variables and chi-square test for categorical variables (\**P*>0.05 significant, \*\**P*>0.001)

MS: multiple sclerosis; NMOSD: Neuromyelitis Optica Spectrum Disorders; OND: other neurological disorders, HC: healthy controls; EDSS: expanded disability status scale; ON: optic neuritis; TM: transverse myelitis; AQP4: aquaporin-4; MOG: myelin oligodendrocyte protein; SEM: standard error mean; <sup>†</sup>: mean±standard error mean; <sup>‡</sup>: range



**Fig 1. Correlation of (A)disease duration with age of onset of MS and NMOSD patients, (B) progression index with disease duration of MS patients, (C) age and antibody type of NMOSD patients. Independent sample T-test, \* $P<0.05$ , \*\* $P<0.001$**

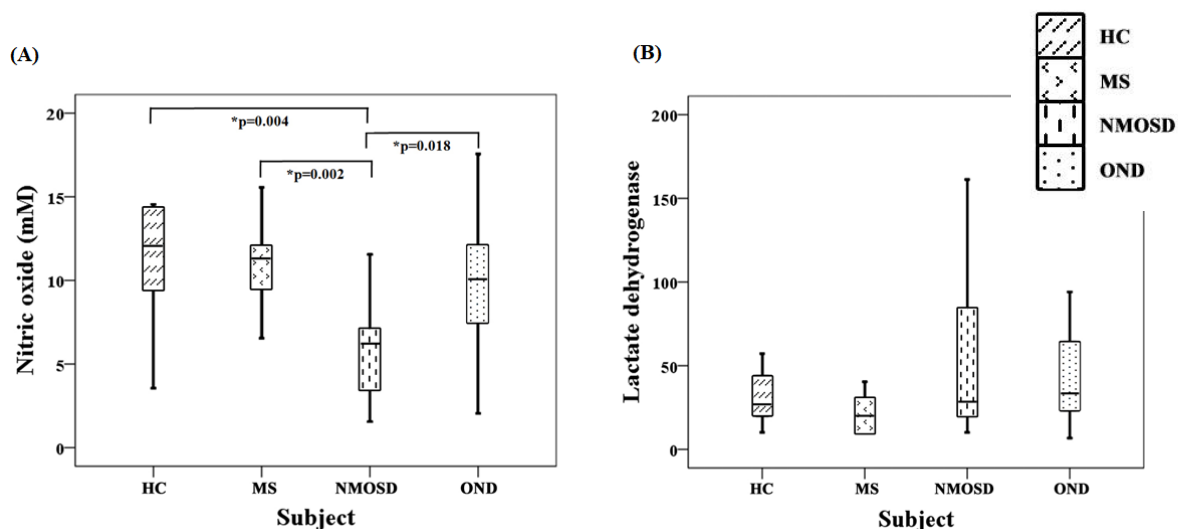
### 3.2 Oxidative parameters

#### 3.2.1 Nitric oxide levels

The levels of Nitric oxide differed significantly between the study groups ( $F_{3,54}=6.362$ ,  $p=0.001$ ) (Figure 2(A)). NOx levels of MS, NMOSD, OND and HC were  $11.01\pm 0.711\text{mM}$ ,  $5.76 \pm 0.819\text{mM}$ ,  $9.97\pm 1.182\text{mM}$  and  $10.92\pm 1.220\text{mM}$  respectively. NOx Levels of MS patients were greater than NMOSD patients ( $P=0.002$ ), and no difference with the HC group ( $P=0.999$ ). Further, levels of NMOSD patients were lower than the OND group ( $P=0.018$ ) and HC group ( $P=0.004$ ).

#### 3.2.2 Lactate dehydrogenase levels

Lactate dehydrogenase levels differed as  $24.29\pm 4.029\text{U/L}$  in MS,  $50.16\pm 12.014\text{U/L}$  in NMOSD,  $43.68\pm 7.756\text{U/L}$  in OND and  $30.91\pm 4.111\text{U/L}$  in HC (Fig 2(B)). No significant difference was observed between the LDH levels of the study groups ( $P=0.07$ ).



**Fig 2. Comparison of (A)Nitric oxide levels and (B)Lactate dehydrogenase of MS & NMOSD patients, OND and Healthy controls. ANOVA, \* $P < 0.05$ , \*\* $P < 0.001$**

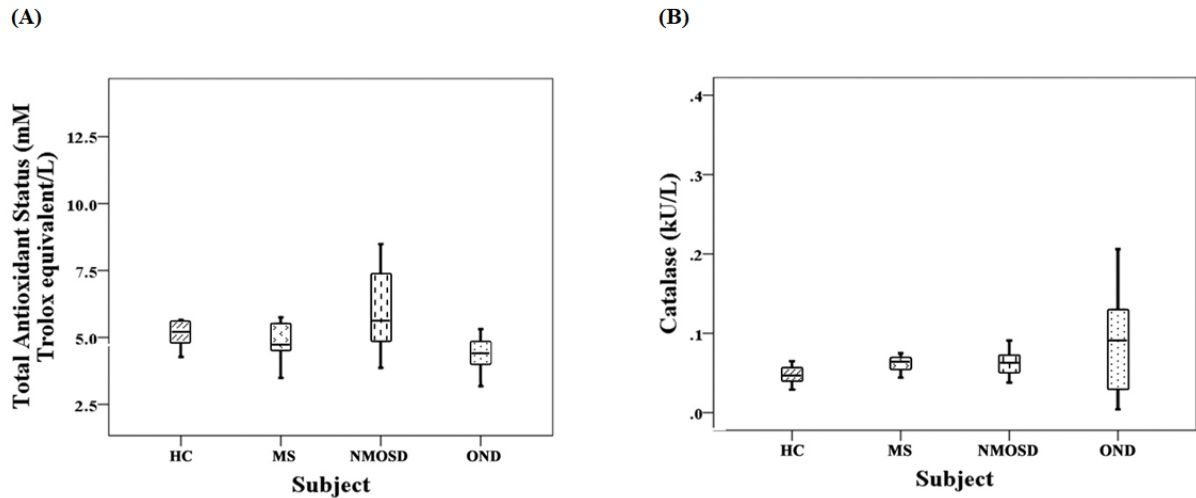
### 3.3 Antioxidant parameters

#### 3.3.1 Total Antioxidant Status

The Total Antioxidant Status of the study groups differed significantly ( $F_{3,52}=3.140$ ,  $P=0.033$ ). Values of TAS levels expressed as mM of Trolox equivalent were  $4.87 \pm 0.295$  in MS,  $6.63 \pm 0.690$  in NMOSD,  $4.64 \pm 0.481$  in OND and  $5.54 \pm 0.438$  in HC (Fig 3(A)). Accordingly, the levels of MS patients were near significantly lower than NMOSD patients ( $p=0.052$ ). However, it is non-significantly greater than healthy controls and lower than OND patients. Levels of NMOSD patients were significantly greater than OND patients ( $P=0.048$ ) and non-significantly greater than healthy controls ( $P > 0.05$ ).

#### 3.3.2 Enzymatic antioxidant –Catalase activity

Catalase activity of MS, NMOSD, OND and HC groups were  $0.060 \text{ kU/L} (\pm 0.003)$ ,  $0.062 \text{ kU/L} (\pm 0.005)$ ,  $0.09 \text{ kU/L} (\pm 0.025)$  and  $0.04 \text{ kU/L} (\pm 0.003)$  respectively (Fig 3(B)). Although non-significant, the levels of catalase activity were higher in MS and NMOSD patients than in healthy controls. A near significant difference was observed between the levels of activity of Catalase enzyme of the study groups overall ( $F_{3,56}=2.537$ ,  $P=0.066$ ), but levels of MS and NMOSD were analogous ( $P=0.999$ ).

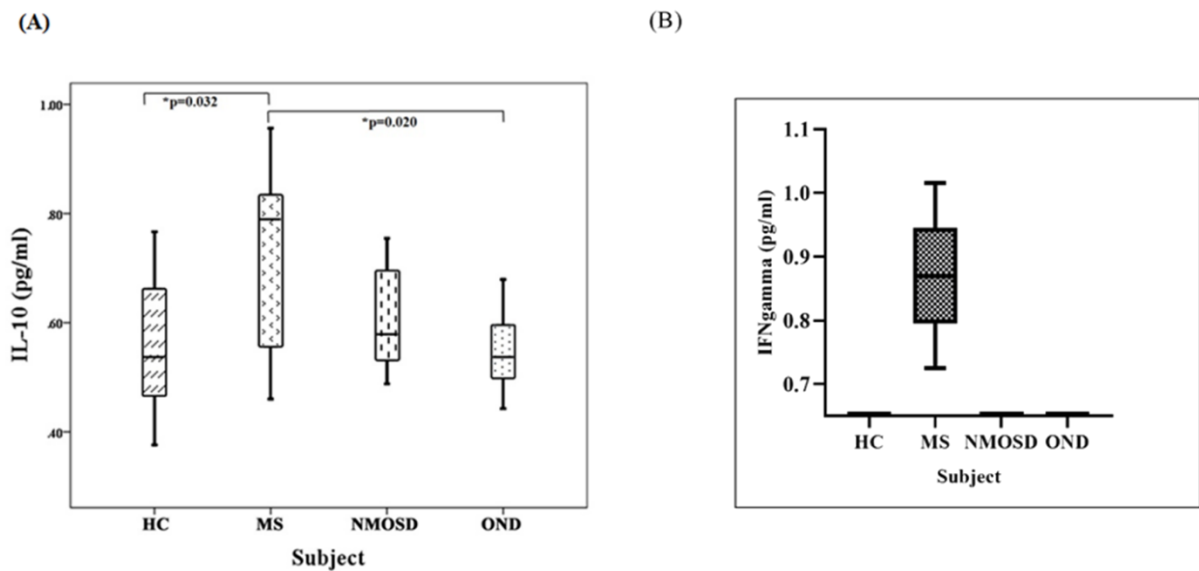


**Fig 3. Comparison of (A) Total Antioxidant Status and (B) Catalase activity of MS & NMOSD patients, OND and Healthy controls. ANOVA, \* $P < 0.05$ , \*\* $P < 0.001$**

### 3.4 Serum cytokine levels

As represented in Fig 4A, the levels of IL-10 showed an overall significant difference between the groups ( $p = 0.013$ ). The highest levels of IL-10 were observed in MS patients, 0.73 pg/ml ( $\pm 0.051$ ) followed by NMOSD patients at 0.61 pg/ml ( $\pm 0.029$ ), HC at 0.56 pg/ml ( $\pm 0.046$ ) and lowest in OND 0.55 pg/ml ( $\pm 0.027$ ). The levels in MS patients were significantly greater than in OND patients ( $P = 0.020$ ) and Healthy Controls ( $P = 0.032$ ).

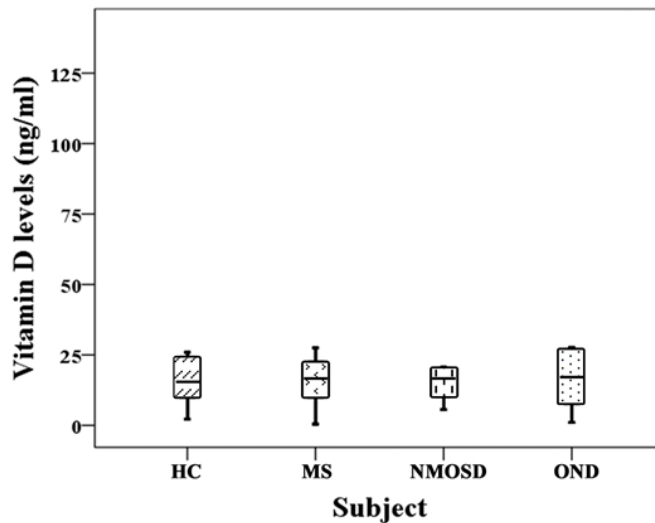
The mean value of IFN $\gamma$  in MS patients was 0.87 pg/ml ( $\pm 0.145$ ), and values of other groups were less than the detection level (Fig 4 (B)).



**Fig 4. Comparison of (A) IL-10 cytokine and (B) IFN  $\gamma$  of MS & NMOSD patients, OND and Healthy controls. ANOVA, \* $P<0.05$ , \*\* $P<0.001$**

### 3.5 Serum Vitamin D levels

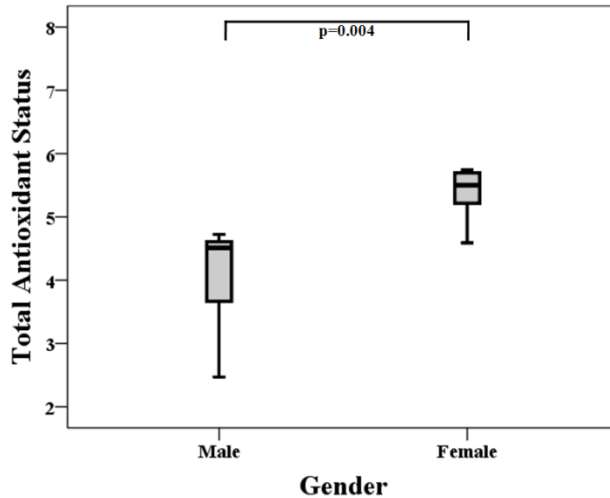
Levels of serum Vitamin D did not show overall significant differences between study groups ( $P=0.491$ ) (Fig 5). The mean values of vitamin D levels of MS, NMOSD, OND and HC were 16.23 ng/ml ( $\pm 2.04$ ), 19.16 ng/ml ( $\pm 3.72$ ), 26.08 ng/ml ( $\pm 8.50$ ) and 16.11 ng/ml ( $\pm 2.52$ ) respectively. MS and NMOSD levels were similar ( $P=0.979$ ), and show a resemblance to healthy controls ( $P=1.000$ ,  $P=0.981$ ).



**Fig 5. Comparison of serum Vitamin D and (D)IL-10 levels of MS & NMOSD patients, OND and Healthy controls. ANOVA, \* $p<0.05$ , \*\* $p<0.001$**

### 3.6 Correlations between the demographic data of participants and serum parameters

In MS patients' NOx and LDH levels did not correlate with any of the demographic and clinical parameters of age, age at onset, disease duration, gender, EDSS scores and progression index. Higher TAS levels were recorded in females than in males ( $p=0.04$ ) (Fig 6) and no other parameters correlated.

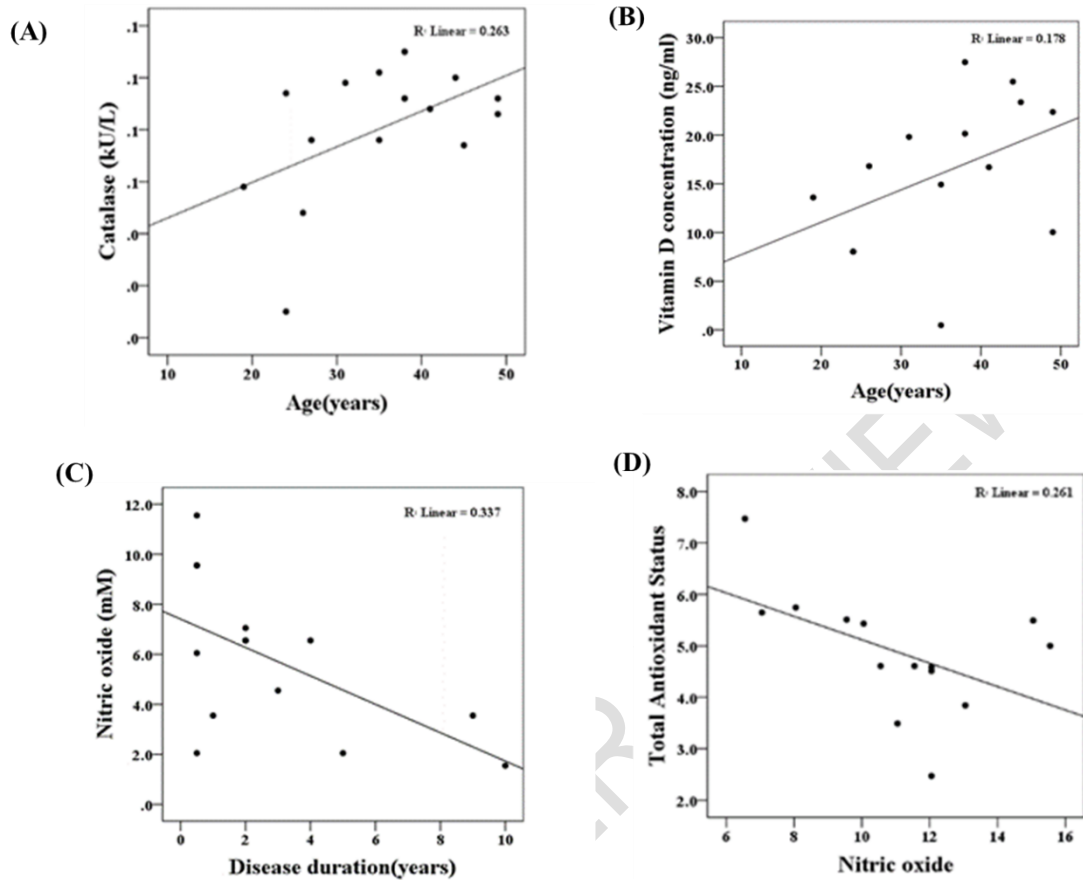


**Fig 6. Gender differences in Antioxidant Status of MS patients. Independent sample T-test, \* $p < 0.05$**

Catalase levels only showed a moderate positive correlation with age ( $r = 0.513$ ,  $p = 0.05$ ) (Fig 7(A)). Vitamin D levels had a near significant moderate positive correlation with age ( $r = 0.513$ ,  $p = 0.0611$ ) (Fig 7(B)) and did not correlate with other parameters.

### 3.7 Correlations between the different disease types and serum parameters

In NMOSD patients TAS and Catalase levels did not show any significant correlation with the parameters age, age at onset, disease duration, gender, disease course and antibody levels. Nitric oxide levels showed a significant moderate negative correlation with disease duration ( $r = -0.581$ ,  $p = 0.029$ ) (Fig 7(C)). Near significant correlation was observed in LDH levels with gender. No other demographic and clinical parameters correlated with the oxidative stress parameters in NMOSD patients. Analysis of correlations of the oxidative stress and antioxidant parameters in MS patients show a significant weak negative correlation between Nitric oxide and Total antioxidant status ( $r = -0.612$ ,  $p = 0.020$ ) (Fig 7(D)). No other correlations were observed. Oxidative stress and antioxidant parameters in NMOSD patients did not show any significant correlations with each other.



**Fig 7. Correlation of (A) catalase levels with age of MS patients; (B) vitamin D levels with age of MS patients, (C) nitric oxide levels with disease duration of NMOSD patients, (D) total antioxidant status with nitric oxide levels of MS patients. Independent sample T-test, \* $p < 0.05$**

The present study serves to provide insight into selected redox parameters and cytokine levels of MS and NMOSD patients in Sri Lanka and its comparison with healthy and disease controls.

The majority of NMOSD patients were observed to be having disease course of optic neuritis than transverse myelitis. Further many were seropositive for MOG antibody rather than AQP4 antibody similar to a recent study in Sri Lanka [11]. The results of this study are comparable with previous studies worldwide that have shown that NMOSD is far more common in females, and is associated with older age than that of MS.

Serum Nitric oxide was found to be non-significantly increased in MS patients than in healthy controls and significantly higher than in NMOSD patients depicting similarity to the previous studies. An extensive literature survey revealed no studies conducted on serum NO<sub>x</sub> levels of NMOSD patients. Nitric oxide contributes to the disease process by direct tissue damage, blocking axonal conduction and inducing axonal degeneration. Any apparent discrepancy in NO<sub>x</sub> levels may be because nitrite and nitrate concentrations in CSF can be highly diluted in serum, and also disturbed by sources like peripheral inflammation and diet and interaction with pharmacotherapy or other methodological differences [26].

LDH is a fermentative enzyme which is present in different isoenzymes in many tissues and body fluids and abnormal levels and types of isoenzyme patterns in cerebrospinal fluid have been reported in MS patients [27]. In the present study, there was no significant difference in serum LDH levels among the study groups however, higher levels were observed in NMOSD patients. Serum LDH levels of MS patients were found to be lower than healthy controls. Serum LDH levels indicate general tissue damage [28] it appears to have more clinical significance when separated into isoenzyme fractions. Hence, it is warranted to assess the isoenzymes profiles for the differential understanding of MS and NMOSD patients.

Measurement of TAS revealed that MS patients have lower antioxidant capacity than healthy controls and NMOSD patients. Also, the TAS of NMOSD patients was observed to be greater than healthy controls. Prior studies have not been conducted on assessing the TAS levels of NMOSD patients. While many previous studies show lower TAS levels in MS patients than in healthy controls, only Acar *et al.*, 2012 [29] revealed significantly higher TAS in MS patients than in control. The TAS can provide information on an individual which may also include those antioxidants not yet known or not easily measured as is the sum of endogenous and food-derived antioxidants of the extracellular fluid. Therefore, the determination of serum TAS level reveals the redox status better than the determination of the level of only one antioxidant in circulation [29].

Catalase enzyme activity was similar in MS and NMOSD patients and showed increased levels than healthy controls. Catalase comprises the first line of oxidative stress defence system against ROS along with other antioxidant enzyme superoxide dismutase [30]. Increased activity of catalase in MS and NMOSD serum can be considered a probable compensatory response for confronting the oxidative stress-mediated damage in MS and NMOSD.

Though the primary nature of MS and NMOSD is yet to be elucidated, it is widely accepted that the demyelination is induced by an immune reaction [31]. It is presumed that MS is driven by Th1 cells which are associated with increased levels of IFN $\gamma$  and IL-12 [32]. Cytokines are the soluble mediators that communicate between the immune cells, organs and their responses [33] Understanding the biases of cytokines response of MS and NMOSD will provide insight into diseases specific biomarkers to understand the pathogenesis and disease-modifying treatments.

Following previous studies, higher levels of serum IFN $\gamma$  were reported in MS patients while IFN $\gamma$  was less than the detection level of the commercial ELISA kit in NMOSD patients. Significant increase of IFN $\gamma$  in NMSOD patients was not reported in other studies [16] and the present study also failed to infer a statistical difference due to the presence of very low levels of IFN  $\gamma$  in NMOSD patients and healthy controls.

We identified that levels of serum IL-10 were highest in MS patients followed by NMOSD patients. This contrasts with previous studies which have indicated lower levels of IL-10 in MS patients in comparison with healthy controls [34]. Our results are comparable with others [35] who have reported IL-10 levels were higher in RR-MS patients. We could not detect a significant difference in IL-10 levels among the MS, NMOSD and HC. On the contrary Pentón-Rol *et al.*, [16] have reported lower IL-10 levels in NMO patients than in HC.

Th1 skewed (Th1/Th2) tendency has been associated with MS relapses patients [31]. Low levels of IL10 are predictive of a relapsing state, and levels are increased during disease remission which correlates with the present study since the patients recruited were in the remission stage [34,36]. Although not significant, IL-10 levels were elevated in NMOSD patients compared to HC and OND depicting similarity to Uzawa *et al.* [19]. However, we

could not assess the ratio of Th1/Th2 of cytokines, which would have otherwise identified the degree of imbalance leading to either a pro-or-anti-inflammatory response overall.

The results of 25-Hydroxyvitamin D levels of MS and NMOSD patients were very similar to that of healthy controls. It is globally recognized that lower vitamin D levels are associated with the risk of developing MS. Majority of vitamin D requirement is synthesized by the skin through the photolysis of 7-dehydrocholesterol upon exposure to sunlight [37]. Therefore, the results of the present study may be attributed to the exposure to sunlight as Sri Lanka is a tropical country having prolonged exposure to sunlight throughout the year. However, additional information in terms of any vitamin D intake by the patients and exposure to the sun is required to get a comprehensive idea of endogenous vitamin D synthesis of patients and healthy subjects.

The correlation of serum parameters with demographic data of patients provides important insights into the differential pathogenesis of MS and NMOSD. Higher levels of TAS in female patients may be accounted for by variations in diet, disease activity, and sex hormones. This could be attributed to variations in testosterone and estrogen hormones that have been shown to correlate with TAS [38]. Multiple sclerosis is recognized to stabilize with time, where the disease progresses rapidly and then reaches a stable condition. This may be a factor that affects the reduction of NOx with time. Further, the inverse correlation of NOx with TAS in MS patients verifies the disruption of oxidant-antioxidant balance leading to increased oxidative status.

#### **4. CONCLUSION**

The present study which has compared the two diseases MS and NMOSD has revealed new insight in terms of oxidative and antioxidant parameters. A major limitation of this study is the low participant number. Since MS and NMOSD patients routinely undergo a complete blood count analysis on admission, oxidant parameters may be used in clinics as practical and reliable prognostic markers of the diseases upon further studies with increased participant numbers and additional parameters. More extensive study in terms of underlying molecular mechanisms would serve to provide broader and deeper knowledge that would pave the way for antioxidant therapy which could be helpful as an immunomodulatory treatment for MS and NMOSD patients thereby increasing their standards of living.

## CONSENT TO PARTICIPATE

Written informed consents were obtained for blood collection and to use medical records.

## ETHICS APPROVAL

The study was approved by the ethics review committee, faculty of medicine, university of Colombo (ec-18-039) and the ethical review committee, the national hospital of Sri Lanka (aaj/eth/com/2018).

## DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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## **ABBREVIATIONS**

anti-MOG- Myelin Oligodendrocyte Glycoprotein

AQP4- Antigen aquaporin-4

CNS-IDDs- Central nervous System Iodopathies

EDSS- Expanded disability status score

FBS- Foetal bovine serum

HC- Healthy controls

IFN $\gamma$ - Interferon gamma

IL- Interleukin

LDH- Lactate dehydrogenase

MOG-Myelin Oligodendrocyte Glycoprotein

MS-Multiple Sclerosis

NMOSD-Neuromyelitis Optica Spectrum Disorders

NOx- Nitric Oxide

OD- optical density

ON- Optic neuritis

OND- Other neurological disorders

PBS- Phosphate buffered saline

PI- Progression index

ROS- Reactive oxygen species

RRMS- Relapsing Remitting Multiple Sclerosis

RT- room temperature

S.E.M- Standard Error Means

TAS- Total Antioxidant Status

TM- Transverse myelitis

TMB- Tetramethyl Benzidine

TNF $\alpha$ - Tumor necrosis factor alpha

UNDER PEER REVIEW