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Original Article

Prevalence of Iron Overload in Patients with Multiple Sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic autoimmune inflammatory condition results in dysfunction of the central nervous system. There are multiple causes linked to the development of MS disease including EBV exposure, vitamin D deficiency, and iron overload. This study aims to examine the frequency of iron overload in all multiple sclerotic patients with reported iron serum levels at the National Guard Hospital (NGHA), Riyadh Center between 2015 and 2021.
Materials and methods: A chart review based cross sectional retrospective study was conducted using the Best Care System Database, developed by NGHA, to estimate the prevalence of iron overload in MS patients. Data were analyzed to identify the correlation between serum iron and ferritin levels with disease severity and activity, which were clinically assessed by the EDSS. The mean findings of blood analysis were also compared between males and females.
Results: The findings of this study showed that there was no significant relationship between disease severity and elevated serum iron and ferritin level, with p-values of 0.130 and 0.856, respectively, 54.1% of the sample had an iron level that was low/very low. The disability score of 20.3% of the sample affects their full daily activities. There was no significant difference between males and females in all means of blood analysis categories as P>0.05.
Conclusion: Our study showed that the degree of disease severity and activity did not significantly correlate with the higher serum iron levels (p-value of 0.130). Further study is needed to investigate the iron level in the active MS plaques of the brain and spinal cord, and assess the severity in accordance to the sites of the specific plaques.

GRAPHICAL ABSTRACT

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Introduction

Multiple sclerosis (MS) is the most prevalent chronic inflammatory, autoimmune, and non-traumatic disease that impairs the central nervous system (CNS). Concerns have been raised due to its unclear underlying pathophysiology. However, environmental, and genetic variables, including Epstein-Barr virus infection, MHC gene alterations, and iron overload, may all play a role in the development of multiple sclerosis. Patients with MS typically present with distinctive symptoms, including visual impairment, numbness, widespread muscular weakness, stiffness, difficulty swallowing, plaque formation, movement restrictions, speech impairment, and sexual dysfunction [1].

There is an increasing incidence and prevalence of MS in young adults in both developed and developing countries [2]. According to a study conducted by Mohammed Aljumah *et al.* [3], 77.5% of patients with multiple sclerosis were under the age of 40 and females were twice as likely as males to have the disease. In addition, Saudi Arabia has the higher annual incidence of MS and lower prevalence comparing to the other countries including those located in Europe and Asia [4].

Iron is one of the crucial elements in the human body which is responsible for multi-systemic functions including oxygen transportation, DNA synthesis, and electron transport. Iron is obtained through both recycling of the RBC (accounting for ~95%) and food intake (accounting for ~5%) [5]. Normal iron serum level in adults is about 60-170 (mcg/dL) [6]. The iron level is tightly regulated according to the body need by hepcidin which is produced by the liver, erythropoiesis level, and inflammatory status of the body. In further details, hepcidin is the main regulatory hormone for the iron serum level in human body as it controls the amount of transported iron by inhibiting ferroprotein which is responsible for transporting the iron in and out of the cells. Hepcidin production is decreased in case of increase in erythrocytes production, loss of blood, or low oxygen saturation. Thus, the ferroprotein expression will not be inhibited, and iron will move out of the cells (such as macrophages) to be transported by Transferrin to the bone marrow or the other tissues in need [7]. Although iron is highly essential for the human body, high level of iron is associated with the formation of free radicals which result in multi-systemic tissue damage by initiating and exacerbating different immune responses, one of which is the damage of myelin that covers the axons of CNS nerves [8]. Demyelination of the axons is highly associated with different neurological diseases, one of which is multiple sclerosis [9].

MS severity is clinically assessed using the Expanded Disability Status Scale (EDSS) developed by a neurologist called John Kurtzke to assess and quantify disability in MS patients and monitor the disability progression over time. The scaling is based on a detailed physical examination by a neurologist. The Expanded Disability Status Scale range from 0 to 10, in which 0-1 indicates (having no physical disability), more than 4 (the patient is disabled), from 7 to 9 (the patient is restricted to wheelchair or bed), and 10 (death from MS) [10].

This study is conducted to investigate the relationship between multiple sclerosis and Iron overload. The aim of this research is to investigate the prevalence of iron overload in all multiple sclerotic patients. The secondary objective of this research is to estimate and assess the relation between multiple sclerosis severity using EDSS and high iron serum level.

Materials and Methods

Study design, settings, subjects, and techniques

The medical records of MS patients were studied in accordance with the following criteria in order to investigate the prevalence of iron overload in multiple sclerotic patients and the association between the disease activity and iron overload. All the data were obtained at once. Hence, the ideal research design is a retrospective cross-sectional study. The target population of this study consisted of all patients who were diagnosed with multiple sclerosis between 2015 and 2021 at the NGHA-Riyadh center or any other

facility, but they were managed and followed up at NGHA. Using the inclusion criteria, the following standards were included in the study: All MS Saudi Arabian patients with recorded iron serum level including all ages and both genders. Furthermore, to ensure that all the individuals who are meeting the requirements for the inclusion criteria are involved, a non-probability (non-randomized) consecutive sampling technique was utilized. Therefore, the estimated sample size for this study was around 374 patients taking into considerations that the expected margin of error is equal to 5%, expected prevalence is 50% (0.5), confidence level is 95%, and the p-value is less than 0.05 according to the Raosoft sample size calculator [11]. However, after cleaning the data, we ended up with 192 participants. The study took place at King Abdulaziz Medical City (KAMC), where effective healthcare services are provided by a group of doctors and specialists. Each neurology and stroke department encounters 12-15 patients on average each day, in addition to 10-15 new neurology consultations [12].

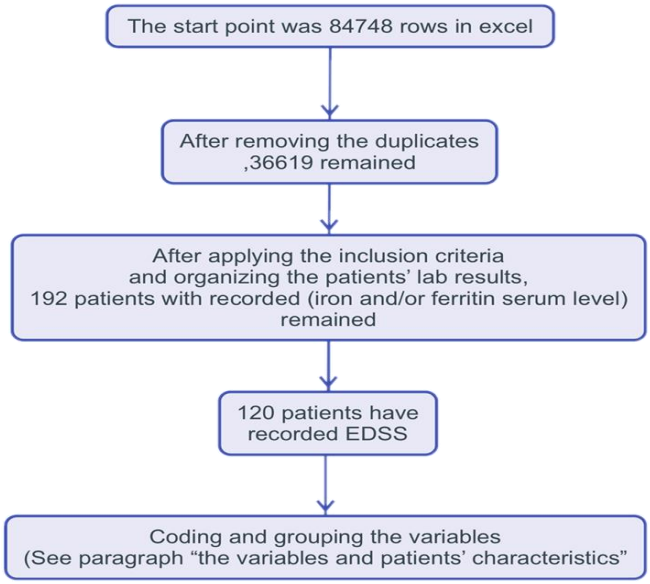

Figure 1: The number of patients included in the study

Table 1: Reference codes

Lab tests reference/coding						
Variable (Code)	Low (1)		Normal range (2)		High (3)	
RBC	<4x10^12/L		4-5.4x10^12/L		>5.4x10^12/L	
WBC	<4x10^9/L		4-11x10^9/L		>11x10^9/L	
Hct	M (<0.42L/L) F (<0.36L/L)		M:0.42-0.54L/L F:0.36-0.54L/L		>0.54L/L >0.54L/L	
Iron	M:<11.6Umol/L F:<9umol/L		M:11.6-31.3umol/L F:9-30.4umol/L		M:>31.3umol/L F:>30.4umol/L	
Ferritin	M:<24ug/L F:<11ug/L		M:24-336ug/L F:11-307ug/L		M:>336ug/L F:>307ug/L	
Variable/code	1 (Normal)		2 (High)		3 (Very high)	
NRBC%	0		>0-<=1		>1	
Patient's general characteristics						
Variables/code	1		2		3	4
Age	<= 16y		17 – 30y		31 – 45y	>= 46y
	1	2	3	4	5	6
BMI	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	>40
Code (0) =missing data						

The study proposal was approved by King Abdullah International Medical Research Center 'KAIMARC' IRB/0475/22.

Study instrument and data collection method

We carried out a review based, cross-sectional study among 192 patients using excel sheet received from KAIMARC. We received 84747 MS patients from KAIMARC in an excel form without our specific inclusion criteria. Upon removing the duplicates as a part of the data cleaning, 36618 remained. From the remaining individuals, we targeted the patients who met our inclusion criteria in which we removed all the patients who have no recorded iron and/or ferritin serum level, 192 patients were finalized to be included in our study, from which only 120 patients had a clinically estimated EDSS. Minimal bias might be related to the missing data regarding iron and ferritin serum level (Figure 1).

The variables and patients' characteristics

Some of the variables of 192 patients including: iron, ferritin, RBC, WBC, and NRBC%, Hct were classified into low, normal, or high coded as 1, 2, and 3, respectively, while the other variables, such as BMI, age, and gender required a specific coding method. Based on our study, the variables were further classified into independent (gender, BMI, and age) and dependent (iron, EDSS, RBC, ferritin, and WBC) variables. Each variable was classified according to its specific range that is different between male and female (Tables 1, 2, and 3 of the results section).

Statistical analysis

Data were coded for entry and analysis using SPSS statistical software package version 24. Data were presented using descriptive statistics in the form of frequencies and percentages. Nominal and ordinal variables were presented in the form of numbers and percentages. Interval and ratio variables were presented in the form of means and standard deviations. Spearman correlation was used to test the relationship between disease severity, serum iron, and ferritin level. Independent sample t-test was used to compare between males and females regarding mean results of blood analysis. The significance level was determined as ($p<0.05$).

Results and Discussion

The aim of this research is to investigate the prevalence of iron overload in all multiple sclerotic patients and the role of iron overload in MS activity and disease severity in NGHA Riyadh Center between 2015 and 2021. The study design was retrospective/ cross-sectional design. Data was collected from 192 patients' records.

Most of the sample (81.3%) were females, while only 18.8% were males. The mean age of the sample was 22 + 12.08. Almost half of the sample (48.4%) aged between 17 and 30 years old. The mean body mass index of the sample was 26.36 + 6.57 (Table 4).

Table 2: Summary of variables and patients' characteristics: EDSS ranking (10)

EDSS	
Score	Clinical presentation
0	Normal function
1	No disability (minimal signs)
2	Minimal disability
3	Moderate disability
4	Relatively severe disability
5	Disability affects full daily activities
6	Assistance required to walk and work
7	Essentially restricted to wheelchair
8	Restricted to bed
9	Bedridden and unable to communicate or eat/swallow
10	Death
M	Missing data

Table 3: The detailed categorization of patients' characteristics and variables based on gender

Variables	Male (36)											
Age (year)	1(0)		2(16)		3(14)				4(6)			
BMI	0(1)	1(3)	2(11)		3(9)		4(7)		5(2)		6(3)	
RBC	1(6)				2(25)				3(5)			
WBC	1(11)				2(21)				3(4)			
NRBC	0(1)		1(33)		2(1)				3(1)			
Iron	0 (4)		1 (19)		2 (13)				3 (0)			
Ferritin	0(8)		1(11)		2(14)				3(3)			
EDSS	0(4)	1(8)	2(1)	3(1)	4(4)	5(1)	6(5)	7(3)	8(0)	9(0)	10(0)	M(9)
Variables	Female (156)											
Age (year)	1(5)		2(77)		3(60)				4(12)			
BMI	0(1)	1(13)	2(61)		3(48)		4(18)		5(11)		6(4)	
RBC	1(22)				2(130)				3(4)			
WBC	1(40)				2(104)				3(12)			
NRBC	0(12)		1(133)		2(9)				3(2)			
Iron	0 (26)		1 (55)		2 (74)				3 (1)			
Ferritin	0(25)		1(26)		2(96)				3(9)			
EDSS	0(32)	1(15)	2(5)	3(5)	4(9)	5(4)	6(9)	7(14)	8(0)	9(0)	10(0)	M (63)

Table 4: Sociodemographic data of the sample (N = 192)

Variable	Number	Percent
Gender Male Female	 36 156	 18.8 81.3
Age (year) >16 17 – 30 31 – 45 < = 45	 5 93 76 18	 2.6 48.4 39.6 9.4
Mean SD	22.00 12.08	
BMI Underweight Normal Overweight Obese Severely obese Morbidly obese	 19 72 54 26 13 8	 9.9 37.5 28.1 13.5 6.8 4.2
Mean SD	26.36 6.57	

This table showed the frequency distribution of the blood analysis results of the sample. The mean red blood cell of the sample is 4.54 + 0.58. The majority of the sample (80.7%) had a normal reading of RBC. The mean NRBC was 0.06 + 0.32. The majority of the sample (86.5%) had a normal NEBC reading. The mean WBC was 6.71 + 3.43. About two thirds of the sample (65.1%) had a normal NEBC reading. The mean Iron level was 9.49 + 7.58. More than half of the sample (54.1%) had either very low or low iron level. Regarding the ferritin level, the mean was 167.12. + 631.66. More than half of the sample (58.3%) had normal ferritin level and 16.1% were not recorded. More than half of the sample (54.1%) had either very low or low iron level (Table 5).

Table 5: Review numbers, frequency, and distribution of blood analysis results of the sample (N = 192)

Variable	Number	Percent
RBC		
Below normal	28	14.6
Normal	155	80.7
Above normal	9	4.7
Mean	4.54	
SD	0.58	
NRBC		
Below normal	13	6.8
Normal	166	86.5
Above normal	13	6.8
Mean	0.06	
SD	0.32	
WBC		
Below normal	51	26.6
Normal	125	65.1
Above normal	16	8.3
Mean	6.71	
SD	3.43	
Iron		
Very low	30	15.6
Low	74	38.5
Normal	87	45.3
Above normal	1	0.5
Mean	9.49	
SD	7.58	
Ferritin		
Below normal	37	19.3
Normal	112	58.3
Above normal	12	6.3
Not recorded	31	16.1
Mean	167.12	
SD	631.66	

Table 6: Frequency distribution of different medications used by the sample (N = 192)

Medication	Number	Percent
Interferon Beta-1a Intramuscular Injection or subc (AVONEX®)	68	35.4
Natalizumab 20 mg/mL Injection, 15 mL Vial	30	15.6
Ocrelizumab 300 mg/10 ml Vial	50	26.0
Fingolimod (Gilenya) Capsule	36	18.8
Teriflunomide (Aubagio) 14 mg FC tablet-NFD	10	5.2
RiTUXimab 10 mg/mL Injection, 50 mL Vial	11	5.7
Dimethyl Fumarate (Tecfidera) 240mg-DR Cap- NFD	4	2.1

More than one third of the sample (35.4) used Interferon Beta-1a Intramuscular Injection or subcutaneous. (AVONEX®). More than one quarter (26.0 %) of the sample used Ocrelizumab 300 mg/10 ml Vial. Only 2.1% of the sample used Dimethyl Fumarate (Tecfidera) 240 mg-DR Cap-NFD (Table 6).

A bit less than a third of the sample (30.7%) had no disability. (2.6%) of the sample presented with disability that affect their full daily activities.

(15.7%) of the sample either needed assistance to walk, and work, or were restricted to wheelchair. More than one third of the sample their disability level was not recorded (Table 7).

There was no statistically significant relationship between disease severity and both iron and ferritin level P = 0.130 and 0.856, respectively (Table 8).

There was no statistically significant difference between males and females in all means of blood analysis categories as p-value more than 0.05 in all blood analysis categories such as RBC, NRBC, WBC, Hemoglobin, Platelet, Iron, Ferritin, and folic acid (P = 0.63, 0.49, 0.83, 0.87, 0.90, 0.79, 0.69, and 0.91, respectively) (Table 9).

We hypothesized that iron overload is associated with a more severe form of the disease. Previous literatures suggest the role of abnormal iron level in developing the neurodegeneration seen in MS [13]. In this study, the results showed that there is no statistical significance of the relationship between increased iron levels and MS severity (p-value=0.130, r-coefficient=0.156 for iron, and p=0.856, r-coefficient= 0.015 for ferritin). For further interpretation, out of 192 patients, 1 female had abnormally high serum iron level and 9 with elevated ferritin level. While in males only 3 showed elevated ferritin level and none of them showed abnormality in serum iron level. Out of 156 female patients, 14 of them had the highest EDSS which is 7, essentially restricted to wheelchair; nevertheless, 63 patients were not clinically assessed for EDSS. On the other hand, out of 36 male patients, the highest score was 7 in only 3 patients while 9 of which had no recorded EDSS. Scientifically, high serum level of iron contributes to an increase in inflammation and oxidative stress. Therefore, it exacerbates the disease process and neurodegeneration.

Table 7: Frequency Distribution of level of disability of the sample (N = 192)

Level of Disability	Number	Percent
No disability (minimal signs)	59	30.7
Minimal disability	6	3.1
Moderate disability	6	3.1
Relatively severe disability	13	6.8
Disability affects full daily activities	5	2.6
Assistance required to walk and work	13	6.8
Essentially restricted to wheelchair	17	8.9
Not recorded	72	37.5

Table 8: Relationship between disease severity and serum iron and ferritin level

Variables	Disease Severity	
	r-coefficient	P-value
Iron	0.156	0.130
Ferritin	0.015	0.856

Table 9: Comparison between Males and Females Regarding Mean Results of Blood Analysis

Blood Values	Male		Female		F	P-value
	Mean	SD	Mean	SD		
RBC	4.58	0.70	4.53	0.56	3.92	0.63
NRBC	0.09	0.50	0.05	0.26	1.97	0.49
WBC	6.81	4.14	6.68	3.25	1.19	0.83
IRON	9.80	6.36	9.43	7.83	2.17	0.79
Ferritin	126.00	241.57	176.54	691.07	0.50	0.69

Moreover, the neurodegeneration process contributes to the exacerbation of iron levels as well [14]. Majority of the sample size in this study are women. In 2020, Fariha Angum demonstrated that “eighty percent of all individuals affected by autoimmune disorders tend to be women due to variation within the sex chromosomes and hormonal changes” [15]. In women with

conditions of iron overload, menstrual period normalizes the iron level because it provides opportunity for iron loss, which may explain why majority of females in this study have normal iron level [16].

A study was proposed by Leslie S. Valberg *et al.* had sample size of 49 patients (31 females and 18 males). 11 patients had abnormalities in iron serum levels, 9 of which were restricted to wheelchair (EDSS of about 7). Even though our study had only 1 female patient who presented with elevated iron level and was restricted to wheelchair (EDSS of 7) [13]. Another study was conducted in 2022 by Hamdi E *et al.* and calculated both global and regional brain iron load. Iron load was compared between 30 MS patients and 15 healthy controls using 3T-MRI. They found that the global range of iron levels was insignificant between the 2 groups. However, regional iron levels showed significant difference between the 2 groups. The study also found that there was no association between global iron level and the clinical presentation, unlike the regional iron level, which showed association with patients' disability status (p-value < 0.039) [17]. Unlike our study that was conducted based upon the iron serum level rather than brain iron load which initially showed no association of EDSS and iron serum level.

Limitations, Recommendations, and Unexpected findings

The iron levels that were recorded in both BEST Care System and excel sheet that we had were very limited and some patients had only one recorded iron serum level. The estimated sample size was about 370, but we were only able to collect 192 patients after applying the inclusion criteria. Thus, it is preferred to acquire a larger number of sample size for any future studies. Moreover, another study that can be conducted as well, which is the regional differences in iron levels in the brain and its correlation to clinical presentation (according to each region).

One major unexpected finding is the low serum iron in male patients. Out of the 36 male patients, 19 had low serum iron level and 11 had low ferritin level which accounts for 52%.

Conclusion

The purpose of this study is to estimate the prevalence of iron overload in MS patients of NGHA-Riyadh Center between 2015 and 2021. Moreover, the Expanded Disability Status Scale (EDSS) of MS patients was obtained to estimate and evaluate the relationship between multiple sclerosis severity and high iron serum levels. There was no significant correlation between disease severity and elevated iron serum levels (p-value 0.130). Out of 36 male patients, none of them had iron overload whereas in 156 female patients, only 1 had iron overload.

Disclosure Statement

No potential conflict of interest was declared by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the manuscript and agreed to be responsible for all the aspects of this work.

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