Caspian Journal of Neurological Sciences
"Caspian J Neurol Sci"

Journal Homepage: http://cjns.gums.ac.ir

Research Paper: Serum Bilirubin Level Changes in Multiple Sclerosis Patients

Mahdi Barzegar[1,2], Vahid Shaygannejad[1,3], Omid Mirmosayyeb[1,2], Samane Valipour[1,2], Roya Akbari[1,2], Sahar Vesal[1], Fereshte Ashtari[1], Leila Dehghani[1], Abdolrasoul Mohammadi[1], Nafiseh Esmaeil[4*]

1. Neurosciences Research Center, Alzahra Institute, Isfahan University of Medical Sciences, Isfahan, Iran
2. Student Research Committee, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Neurology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Use your device to scan and read the article online

Citation: Barzegar M, Shaygannejad V, Mirmosayyeb O, Valipour S, Akbari R, Vesal S, et al. Serum Bilirubin Level Changes in Multiple Sclerosis Patients. Caspian J Neurol Sci. 2018; 4(1):30-34. https://doi.org/10.29252/NIRP.CJNS.4.12.30

Running Title: Bilirubin Levels in Multiple Sclerosis

doi: https://doi.org/10.29252/NIRP.CJNS.4.12.30

Bullet Points:

- Bilirubin is an endogenous antioxidant and a neuroprotective element in human.
- Bilirubin level is lower in MS patients and its deficit may play role in neuropathology of MS.

Article History:
Received: 30 Nov 2017
Accepted: 19 Dec 2017
Available Online: 01 Jan 2018

ABSTRACT

Background: Multiple Sclerosis (MS) is a demyelinating disorder of the Central Nervous System (CNS). In addition to the role of immune mediated mechanisms, oxidative and nitrosative stress also play an important role in neuropathology of MS. Bilirubin as an endogenous antioxidant has neuroprotective effects; however few studies have assessed the association between serum bilirubin level and MS.

Objectives: To evaluate serum level of bilirubin in MS patients.

Materials and Methods: Serum samples were collected from participants who referred to Kashani MS clinic in Isfahan between July 2016 and July 2017. A total of 80 MS patients (67 females and 13 males) and 94 Healthy Control subjects (HCs) (62 female and 32 male) enrolled. Compression of bilirubin levels between MS patients and HCs was done with covariance analysis. Regression analysis was used to assess the relation between bilirubin concentration and EDSS. SPSS software version 17.0 for Windows (SPSS, Chicago, IL, USA) was used.

Results: The level of Direct bilirubin (Dbil) was significantly lower in MS patients compared with HCs (P=0.02). Otherwise the serum concentration of Total bilirubin (Tbil) and in Direct bilirubin (Ibil) were higher in MS patients, but it was not statistically significant. There was a negative correlation between Extended Disability Status Scale (EDSS) and bilirubin levels (Tbil, Dbil and Ibil) but it was not significant.

Conclusion: Bilirubin level is lower in MS patients and deficit of its antioxidant level may play role in neuropathology of MS.

Keywords: Bilirubin, Multiple sclerosis, Antioxidants

*** Corresponding Author:**
Nafiseh Esmaeil, PhD
Address: *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
Tel: *+98 (311) 37929097* , ***Fax****: +98 (311) 37929031*
E-mail: *nafesm5@gmail.com*

Introduction

Multiple sclerosis (MS) is a demyelinating immune disease of Central Nervous System (CNS) and a cause of disability in middle-aged people [1, 2]. It is characterize by clinical attacks, inflammation, demyelination and plaques in the brain and spinal cord [3]. In addition to role of immune mediated mechanisms in neuroimmunological disorders such as MS, recent findings suggest that oxidation of brain phospholipids and proteins have an important role in pathogenesis of these disorders [3-5]. Over production of the Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) that cause oxidative and nitrosative stress plays a main role in neuropathology of Multiple Sclerosis (MS) during the disease onset and its promotion [6, 7].

Macrophages and microglial cells generate ROS and RNS during CNS inflammation. These cells mediate demyelination and neurodegeneration through apoptosis of oligodendrocytes and also oxidize cardinal cellular components such as DNA molecules, lipids and proteins [8, 9]. This pathological condition appears as a result of impaired in the redox homeostatic mechanisms and reducing in the endogenous antioxidants such as bilirubin levels. Bilirubin as an endogenous antioxidant plays critical role in the control of oxidative stress and ROS production [10]. It is an endogenous product of heme breakdown. Senescent heme degrades by hemeoxygenase inside macrophages of the spleen and metabolize to biliverdin, carbon monoxide and iron. Afterward, biliverdin is converted to bilirubin by biliverdin reductase [11, 12].

Two decades, researchers have indicated bilirubin is an important physiological neuroprotective antioxidant. Experimental researches on rats have displayed bilirubin with a protective effect against traumatic brain injuries, cerebral ischemia damage and intracerebral hemorrhage [13, 14]. Bilirubin protects Blood Brain Barrier (BBB) from immune cell invasion and scavenges the ROS in spinal cord lesions and protects CNS from damage in MS patients. Additionally in animal model of MS, therapeutic effect of bilirubin in long term has been more effective than dexamethasone and other antioxidants [15]. Liu.et al have explained the immunomodulatory role of bilirubin in the Experimental Autoimmune Encephalomyelitis (EAE) as a model for multiple sclerosis. Bilirubin as an immunomodulator, suppresses both polyclonal and antigen-specific T cell responses and also induces anergy against reactive CD4+T cells via inhibition of costimulatory signaling [16]. Despite decisive antioxidative and immunmodulatory properties of bilirubin, there is inadequate research that surveys its role in MS patients. So in the present study, we assessed the levels of bilirubin (total, indirect and direct), in order to determine the changes of this natural endogenous antioxidant in the serum of MS patients compared with healthy control subjects We also looked for a possible association between bilirubin serum concentrations and clinical characteristics of disorder and its progression.

Materials and Methods

Serum samples were collected from 174 individuals comprising 80 MS patients who attended to Kashani hospital MS clinic, Isfahan, Iran and 94 healthy controls. Venous blood was collected in the morning after an overnight fast from patients and Healthy Controls (HCs) for measurement of serum bilirubin. Serum total bilirubin (normal range: 0.1-1.3 mg/dl), Direct bilirubin (normal range: 0.1-0.4 mg/dl) and inDirect bilirubin (normal range: 0.1-0.9 mg/dl) concentration were measured using dichloroaniline method. At the same time, Alanine Transaminase (ALT) (normal range: 1-31 mg/dl for female and 1-41 mg/dl for male) and Aspartate Transaminase (AST) concentrations (normal range: 1-38 mg/dl for female and 1-40 mg/dl for male) were also measured with International Federation of Clinical Chemistry (IFCC) method. Four subjects (One patient and 3 HCs) were excluded from the study because of abnormal liver enzymes. Patient's data were compared with 94 HCs. Demographic and clinically information of subjects are presented and summarized in Table 1. All patients had definite MS according to the criteria of McDonald et al. 2010 [17]. The severity of disease in patients was evaluated by using the Expanded Disability Status Scale (EDSS) score [18].

Exclusion criteria were liver enzyme abnormality, other neurological and autoimmune diseases, receiving of immunosuppressive or interferon medication at least 6 months prior to study entry. The study was approved by Vice President of research of Isfahan University of Medical Sciences (project number: 293398) and written informed consents were obtained from all subjects before enrollment.

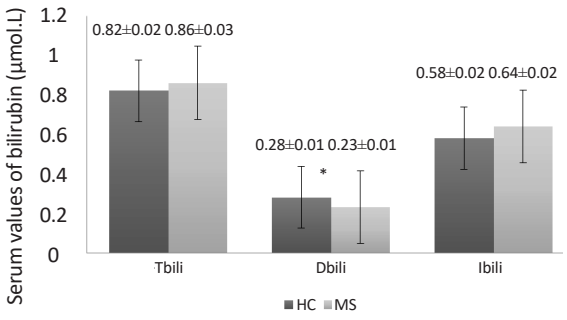
The comparisons of all types of bilirubin concentrations between MS patients and HC subjects were examined with covariance. The relation between sex and age with serum levels of bilirubin in MS patients and HCs was assessed with covariance analysis. The relation between bilirubin concentration in MS patients and EDSS was analyzed using regression analysis. All statistical calculations were done using the SPSS 17.0 for Windows (SPSS,

Chicago, IL, USA). All data for both groups are presented as mean (SD). The P<0.05 was considered as significant.

Results

The basic demographic and clinical characteristics of the study subjects are given in brief version in Table 1. There was no difference between two groups in terms of gender and age (P>0.05). The mean EDSS of patients were 2.04±1.3. The serum concentrations of Direct bilirubin (Dbil) were lower in MS patients compared to healthy controls (P<0.05). Tbil and Ibil levels were higher in MS patients than those observed in healthy controls, but it was not statistically significant (Figure 1).

There was no statistical difference in Tbil, Dbil, and Ibil levels between female healthy controls and male healthy controls. But Tbil and Ibil levels in female MS patients were significantly lower compared to male MS patients. The comparison of Tbil, Dbil, and Ibil levels between the same genders in different groups showed that Tbil and Ibil levels were higher in male and female MS patients than those observed in healthy controls of the same gender, but it was not statistically significant (Table 2). There was a negative correlation between EDSS of MS patients and bilirubin levels, but it was not statistically significant



CJNS

Figure 1. The serum values of total, indirect and Direct bilirubin (µmol.L)

Tbil: Total bilirubin; Ibil: In Direct bilirubin; Dbil: Direct bilirubin serum values; HC: Healthy Control; MS: Multiple Sclerosis patients. Bars in the graph represent mean±SD of total, indirect and Direct bilirubin serum values. *P<0.05 HC vs. MS (for Direct bilirubin)

(P>0.05). There was no correlation between disease duration and bilirubin levels in MS patients (P>0.05).

Discussion

In the present study, we observed significantly lower serum concentration of Dbil in MS patients. But we found higher levels of Tbil and Ibili in male and female MS pa-

Table 1. Demographic characteristics of healthy controls and multiple sclerosis patients

Subjects	Number	Age (Years)	Female/Male	Disease Duration (Years)
Healthy controls	94	31.33±6.4	62/32	-
MS patients	84	32.73±8.5	67/13	5.7±5

CJNS

Table 2. Serum bilirubin levels in male and female patients, healthy controls and total subjects (mean±SD; µmol/L)

Subjects		Mean±SD (µmol/L) Female	Mean±SD (µmol/L) Male	Range (Imol/L)	P*
All participants	Tbill	0.79±0.02	0.89±0.03	0.1-1.30	0.01*
	Dbill	0.23±0.01	0.27±0.01	0.1-0.4	0.10
	Ibill	0.56±0.01	0.67±0.03	0.3-1.0	0.02*
Healthy controls	Tbill	0.77±0.18	0.85±0.37	0.1-1.30	0.18
	Dbill	0.26±0.05	0.3±0.3	0.1-0.4	0.23
	Ibill	0.53±0.15	0.6±0.32	0.3-1.0	0.17
MS patients	Tbill	0.84±0.05	1.049±0.08	0.4-1.20	0.01*
	Dbill	0.21±0.01	0.23±0.01	0.1-0.4	0.15
	Ibill	0.59±0.02	0.78±0.05	0.3-1.0	0.005*

CJNS

* P-value of male versus vs. female in multiple sclerosis and healthy controls groups

Tbil: Total bilirubin; Ibil : In direct bilirubin; Dbil: Direct bilirubin serum values

tients compared to healthy controls of the same gender. Some of previous studies have suggested patients with MS have a low level of serum bilirubin that our findings were inconsistent with these studies [19, 20].

To our knowledge, major limitations in previous studies have been a small number of patients included. Increasing of ROS activity by oxidative stress leads to nerve injury and may simultaneously cause an overuse of bilirubin. Although bilirubin concentrations in sera might be reduced as a result of an over consumption of bilirubin by produced ROS cleaning, but increasing of bilirubin in male and female MS patients in our study may be secondary for compensation of bilirubin over-consumption [21]. Liu et al. have suggested that increasing of bilirubin levels closely related to reduced neuroinflammation and better clinical manifestation of experimental disease [15]. Changes in activity of key enzymes which are involved in breakdown of porphyrin ring maybe effects on bilirubin production. Hemeoxygenase enzymes have key role in heme catabolism and yield equimolar amounts of biliverdin that is quickly converted by biliverdin reductase into bilirubin. Inducible Hemeoxygenase (HO-1) which induce by high levels of ROS in MS patients have antioxidative and anti-inflammatory properties, while constitutive Hemeoxygenase (HO-2) doesn't have such properties [22, 23].

Many studies have shown enhanced HO-1 expression in residential CNS cells such as astrocytes, reactive microglia, and infiltrated macrophages in animals suffering from EAE. Residential cells in CNS play main role in neuroinflammation and increasing of HO-1 in these cells underline contribution of these cells in neuroinflammation which maybe mediated by bilirubin and its metabolism modulator enzymes [24, 25]. Also it should be noted that, in addition to antioxidant activity bilirubin has immunimodulatory activities such as inhibition of complement cascade and inhibition of antibody dependent cell-mediated cytotoxic activities of lymphocytes [26, 27]. Therefore, we should consider other activities of bilirubin might also be involved in MS pathogenesis and increasing of bilirubin in MS patients probably related to its other activities. It is so complicated to address when bilirubin concentration is being reduced or increased in MS patients. So it is either basically increased by virtue of its protective role against oxidants.

Furthermore, in the present study we found a negative correlation between EDSS of MS patients and bilirubin levels (although it was not significant), which is in agreement with all facts that mentioned above and emphasizes the positive effects of antioxidants on improving of MS.

We found that bilirubin levels (Tbil and Ibil) were significantly lower in female than in male patients, which is in agreement with similar studies [10, 6]. Because the prevalence of MS is higher in women, whether lower endogenous antioxidants levels are related to higher incidence of this disease among females is still unknown. Further studies with larger samples are necessary to judge how bilirubin levels relate to MS disease and the gender of patients.

Conclusion

The study concludes that bilirubin levels in MS patients probably are related to its both antioxidant and immunomodulatory functions. However, more controlled studies are needed in order to investigate the role of bilirubin and its mode of action in MS disease.

Acknowledgements

Isfahan University of Medical Sciences has financially supported the present paper. We thank our patients for their collaboration. Also the authors would like to thank the efforts of the staff at the MS clinic in Kashani hospital. The neurosciences research center, Alzahra hospital, Isfahan, Iran has supported this study financially and so we acknowledge this gesture.

Conflict of Interest

The authors have no conflicts of interest.

References

[1] Mahad D, Ziabreva I, Lassmann H, Turnbull D. Mitochondrial defects in acute multiple sclerosis lesions. Brain. 2008; 131(7):1722–35. doi: 10.1093/brain/awn105

[2] Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis The 2013 revisions. Neurology. 2014; 83(3):278-86. doi: 10.1212/WNL.0000000000000560

[3] Perluigi M, Coccia R, Butterfield DA. 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: A toxic combination illuminated by redox proteomics studies. Antioxid Redox Signal. 2012; 17(11):1590–609. doi: 10.1089/ars.2011.4406

[4] Adibhatla RM, Hatcher JF. Lipid oxidation and peroxidation in CNS health and disease: From molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2010; 12(1):125–69. doi: 10.1089/ars.2009.2668

[5] Lassmann H. Mechanisms of inflammation induced tissue injury in multiple sclerosis. J Neurol Sci. 2008; 274(1-2):45–7. doi: 10.1016/j.jns.2008.04.003

[6] Ljubisavljevic S, Stojanovic I, Vojinovic S, Milojkovic M, Dunjic O, Stojanov D, et al. Association of serum bilirubin and uric acid levels changes during neuroinflammation in patients with initial and relapsed demyelination attacks. Metab Brain Dis. 2013; 28(4):629–38. doi: 10.1007/s11011-013-9409-z

[7] Peng F, Yang Y, Liu J, Jiang Y, Zhu C, Deng X, et al. Low antioxidant status of serum uric acid, bilirubin and albumin in patients with neuromyelitis optica. Eur J Neurol. 2011; 19(2):277–83. doi: 10.1111/j.1468-1331.2011.03488.x

[8] Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: The need for effective antioxidant therapy. J Neurol. 2004; 251(3):261–8. doi: 10.1007/s00415-004-0348-9

[9] Fischer MT, Wimmer I, Höftberger R, Gerlach S, Haider L, Zrzavy T, et al. Disease-specific molecular events in cortical multiple sclerosis lesions. Brain. 2013; 136(6):1799–815. doi: 10.1093/brain/awt110

[10] Acar A, Ugur Cevik M, Evliyaoglu O, Uzar E, Tamam Y, Arıkanoglu A, et al. Evaluation of serum oxidant/antioxidant balance in multiple sclerosis. Acta Neurol Belg. 2012; 112(3):275–80. doi: 10.1007/s13760-012-0059-4

[11] Kapitulnik J. Bilirubin: An endogenous product of heme degradation with both cytotoxic and cytoprotective properties. Mol Pharmacol. 2004; 66(4):773–9. doi: 10.1124/mol.104.002832

[12] Choi AM, Alam J. Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. Am J Respir Cell Mol Biol. 1996; 15(1):9–19. doi: 10.1165/ajrcmb.15.1.8679227

[13] Chang EF, Wong RJ, Vreman HJ, Igarashi T, Galo E, Sharp FR, et al. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. J Neurosci. 2003; 23(9):3689-96. PMID: 12736340

[14] Wang J, Zhuang H, Doré S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage. Neurobiol Dis. 2006; 22(3):473–6. doi: 10.1016/j.nbd.2005.12.009

[15] Liu Y, Zhu B, Wang X, Luo L, Li P, Paty DW, et al. Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis. J Neuroimmunol. 2003; 139(1-2):27–35. doi: 10.1016/s0165-5728(03)00132-2

[16] Liu Y, Li P, Lu J, Xiong W, Oger J, Tetzlaff W, et al. Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. J Immunol. 2008; 181(3):1887–97. doi: 10.4049/jimmunol.181.3.1887

[17] Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011; 69(2):292–302. doi: 10.1002/ana.22366

[18] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurol. 1983; 33(11):1444–52. doi: 10.1212/wnl.33.11.1444

[19] Peng F, Deng X, Yu Y, Chen X, Shen L, Zhong X, et al. Serum bilirubin concentrations and multiple sclerosis. J Clin Neurosci. 2011; 18(10):1355–9. doi: 10.1016/j.jocn.2011.02.023

[20] Deng J, Liang XM, Zhang XL, Ling SQ, Yang TT, Li M, Peng FH. Relationship between serum bilirubin levels and optic neuritis. Chin Med J. 2013; 126(17):3307-10. PMID: 24033954

[21] Maher P, Schubert D. Signaling by reactive oxygen species in the nervous system. Cell Mol Life Sci. 2000; 57(8-9):1287–305. doi: 10.1007/pl00000766

[22] Ljubisavljevic S, Stojanovic I, Vojinovic S, Stojanov D, Stojanovic S, Cvetkovic T, et al. The patients with clinically isolated syndrome and relapsing remitting multiple sclerosis show different levels of advanced protein oxidation products and total thiol content in plasma and CSF. Neurochem Int. 2013; 62(7):988–97. doi: 10.1016/j.neuint.2013.02.025

[23] Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: From basic science to therapeutic applications. Physiol Rev. 2006; 86(2):583–650. doi: 10.1152/physrev.00011.2005

[24] Emerson MR, LeVine SM. Heme oxygenase-1 and NADPH cytochrome P450 reductase expression in experimental allergic encephalomyelitis. J Neurochem. 2008; 75(6):2555–62. doi: 10.1046/j.1471-4159.2000.0752555.x

[25] Ljubisavljevic S, Stojanovic I, Pavlovic R, Stojnev S, Stevanovic I, Sokolovic D, et al. The reduced glutathione and S-nitrosothiols levels in acute phase of experimental demyelination – Pathophysiological approach and possible clinical relevancy. Neuroscience. 2012; 219:175–82. doi: 10.1016/j.neuroscience.2012.05.062

[26] Arriaga SM, Mottino AD, Almará AM. Inhibitory effect of bilirubin on complement-mediated hemolysis. Biochim Biophys Acta. 1999; 1473(2-3):329–36. doi: 10.1016/s0304-4165(99)00201-9

[27] Větvička V, Miler I, Šíma P, Táborský L, Fornůsbk L. The effect of bilirubin on the fc receptor expression and phagocytic activity of mouse peritoneal macrophages. Folia Microbiol. 1985; 30(4):373–80. doi: 10.1007/bf02927593