International Research Journal of Public and Environmental Health Vol.5 (7),pp. 114-120, November 2018
Available online at https://www.journalissues.org/IRJPEH/
https://doi.org/10.15739/irjpeh.18.015

ISSN 2360-8803

Original Research Article

Effect of heavy metals and sialic acid in multiple sclerosis

Received 21 September,2018 Revised 12 November, 2018 Accepted 16 November, 2018 Published 23 November, 2019

Servin Yeşil Günal*[1], Hikmet Saçmacı[2], Şerife Saçmacı[3], Meral Mirza[4] and Funda İpekten[5]

[1]Erciyes University, Vocational Health College 38039, Kayseri, Turkey.
[2]Department of Neurology, Bozok University of Medicine, 66200, Yozgat Turkey.
[3]Department of Chemistry, Erciyes University of Faculty of Science 38039, Kayseri, Turkey.
[4]Department of Neurology, Erciyes University of Medicine 38039, Kayseri Turkey.
[5]Department of Biostatistics, Erciyes University of Medicine 38039, Kayseri Turkey.

Corresponding Author Email: sgunal@erciyes.edu.tr

Tel.: +90 352 437 52 79

The aim of this study is to investigate the effect of heavy metals, sialic acid and other environmental factors in patients with multiple sclerosis. Fifty-two multiple sclerosis patients and 41 healthy volunteers as the control group were included in the study. Fasting saliva and urine samples were taken to determine sialic acid and heavy metals respectively.The levels of Ag ($p<0.001$), Al ($p<0.001$), As ($p<0.001$), Ba ($p<0.05$), Cd ($p<0.005$), Cs ($p<0.001$), Cu ($p<0.05$), Mn ($p<0.05$), Ni ($p<0.001$), Pb ($p<0.001$), Rb ($p<0.05$), Sr ($p<0.05$), and sialic acid levels ($p<0.001$) were found significantly higher, Ti ($p<0.05$) and Fe ($p<0.05$) lower in MS patients compared to the control group. There was a significant relationship between sialic acid, heavy metals levels, vegetable consumption, bowel habits and multiple sclerosis group. In this study, it was found that high levels of sialic acid and heavy metals, less vegetable consumption and bad bowel habits were risk factors for multiple sclerosis.

Key words: Vegetable consumption, heavy metals, multiple sclerosis, sialic acid, bad intestinal habits.

INTRODUCTION

Multiple sclerosis (MS) is a progressive, demyelinating, autoimmune disease of the central nervous system which often develops between ages 20 and 40. It is characterized by acute attacks and remissions (Giacoppo et al., 2014). According to world health organization reports in 2008; actually 2–2.5 million with multiple sclerosis disease were recorded in the world (World Health Organization 2008).

MS is a disease triggered by environmental factors that act on a genetically susceptible host. The nutrition, heavy metals, pesticides, food additives, infections, vaccines and trauma of the head and vertebrae are among environmental factors. The disease occours in certain geographic regions, and developed countries commonly (Giacoppo et al., 2014; Günal et al., 2013).

Heavy metals are taken from drinking water, inhalation of dusts, direct contact or by ingestion of agricultural foods (Giacoppo et al., 2014; Eliaeson et al., 2008). Metals, biological importance, such as copper (Cu), cobalt (Co), chromium (Cr), manganese (Mn), selenium (Se), iron (Fe) and zinc (Zn) play a role in plenty of cell functions such as cell division, electron transfer and ATP production (Giacoppo et al., 2014). Furthermore iron, copper and zinc play a role in production of myelin and neurotransmitters responsible for synaptic transmission (Iranmanesh et al., 2013; Johnson et al., 2000). However, these metals can cause diseases if they are lower or higher than the concentration required for biological functions (Johnson 2000). Exposure to heavy metals such as arsenic (As), lead (Pb), aluminum (Al), nickel (Ni) and mercury (Hg) are known to play an important role in the development of various diseases such as allergic reactions, skin lesions, impaired renal functions, cancer and neurological disorders (Giacoppo et al., 2014; Jarup 2003).

Sialic acid (Sia) is a nine-carbon sugar derived from neuraminic acid and has a negative electrical charge. Sia attaches to the N-terminal ends of glycoproteins or glycolipids (Varki, 2009).The human central nervous system has the highest Sia concentrations (Wang 2009, Wang et al., 2003). Especially there are 20 times more Sia on the surface of the neuronal cell membrane than other membranes (Wang 2009, Wang et al., 2001). Because of their position on the cell surface, these acidic molecules cover the macromolecules and the cells, making them unable to be recognized by the immune system and protect them from enzymatic and immunological attacks (Schauer 2009).They have also very important role in the development of brain tissue, in the regulation of the action potential, in the formation of the synapses, in maintaining the nerve cell adhesion, in the formation of myelin sheath, in programmed cell death, in inflammation and in antioxidant reactions (Wang 2009, Varki 2008). The disappearance of Sia from the cell surface causes the cell to lose its properties and thus the cells are vulnerable to immunological attact. When Sia moves away, macrophages perceive this as a "eat" signal (Varki 2008).

The aim of this study is to investigate the effect of heavy metals, sialic acid and other environmental factors in patients with multiple sclerosis.

MATERIAL AND METHODS

The study was conducted at the Erciyes University Medical Faculty Hospital, Neurology Policlinics between February and April 2015. This study had been approved by the hospital's ethical committee with the following approval number and date: 2014/225, 04.04.2014. Fifty-two (40 females and 12 males) MS patients were included in the study. Thirty five patients were relapsing-remitting, 9 patients secondary progressive and 8 patients were primary progressive of MS patients. All of the patients were using interferon. Patients who had had an attack before the beginning of the study, were being treated with corticosteroids, had history of other diseases and were using iron and other vitamins were not involved in the study.

Control group was chosen among the healthy volunteers (28 females and 13 males) working in the hospital's blood donation unit, similar age and sex properties with the patient group. Forty-one people who had not have a history of MS neither himself/herself nor in his/her family, a history of renal and liver disease, cancer and diabetes, using lithium, any vitamins, minerals and not vegetarians.

The questionnaire was administered to all the participants, following a preliminary trial, by the same physician using the face-to-face technique. The questionnaire consisted of items related to the participants' age, gender, birthplace, nutritional and bowel habits, occupation, smoking, history of MS in the relatives, dental fillings and childhood diseases.

After the participants answered the questions in the questionnaire, saliva and urine samples were taken after an overnight fasting.

The urine samples of the participants were collected into 100 ml sterile polythene tubes and sent to the laboratory for analysis. Urine samples were stored at -20 °C until analysis.

Simultaneously, the participants were asked to rinse their mouth with water before saliva collection and waited 5 minutes then the specimen was collected into a sterile (2 mL) plastic tube. During the intake of saliva samples they did not use any agent that increases the saliva rate or stimulates saliva secretion. Samples were stored at -20 ° C until analysis.

Analysis of samples

Solubilized urine samples collected from MS patients in the control group and for the absence of any contamination, the Berghoff model MWS-3 was dissolved in the high pressure microwave digestion apparatus resistant Teflon containers. Suprapur high purity acid for solubilization (5 mL of concentrate HNO_3) was used. Examples after solubilization, $\mu g\ L^{-1}$ level of the detection limit for the determination of heavy metal levels were used ICP-MS (Model Agilent 7500A), flame photometer for sodium and potassium (Jenway marka), and high concentration levels for the determination of Ca and Mg is to PerkinElmer model 800 flame atomic absorption spectrometry aanalyst (Norwalk, CT, USA), deuterium background correction system using in an air-acetylene mixture were determined using the calibration techniques. When the analyte concentration in real samples was carried out outside the calibration range, if necessary, sample dilution was done.

Which is a standard method for determination of sialic acid found in saliva samples, sialic acid (NANA) ab83375 Assay Kit was used. For this, 20 µL of saliva samples were taken. Each sample is added to 80 µL hydrolysis reagent and heated to 80°C water bath and incubated for one hour. It was cooled to room temperature and after cooling 20 µL of sample was added to the neutralization reagent. It was stirred with shaking by hand. It was centrifugation at 4000 rpm. 50 µL of the sample including 10 µL standard and 450 µL of purified water were mixed and thus standard solution was prepared from the mixture obtained. In each sample, 93 µL of the assay buffer, 1 µL dye reagent and 1 µL enzyme were added. It was kept in the cold cabinet. After standing again at room temperature for 60 minutes the optical density determinations were performed with the calibration method of the Epoch Microplate Spectrophotometer at 570 nm. All methods were performed according to standard procedures.

Statistics

All statistical analysis were performed using the SPSS

Table 1. Socio- demographic characteristics of controls and multiple sclerosis patients

Variable	MS patients % (n= 52)	Controls % (n= 41)	*p*
Age (mean±SD)	37.86±10.4	36.17±12.6	0.481
Gender			
Female	76.9	68.3	0.480
Male	23.1	31.7	
Birthplace			
Rural	30.8	17.1	
City	26.9	61.0	0.08
Out of province rural	30.8	14.6	
Out of province city	11.5	7.3	
Marital status			
Married	77.0	39.0	
Single	19.2	58.6	<0.001
Widow	3.8	2.4	
Educational status			
Primary school	61.5	26.8	
High school	21.2	12.2	<0.001
University	17.3	61.0	
Occupation			
Housewife	59.6	26.8	
Unemployed	11.5	9.8	
Officer	5.8	14.6	
Tradesman	3.8	39.0	<0.001
Retired	5.8	2.4	
Worker	7.7	7.3	
Student	5.8	-	

version 19.0 packet program. Histogram and q-q plots were examined, Shapiro-Wilk's test was applied to assess the data normality. Levene test was used to test variance homogeneity. To compare the differences between groups, Pearson $\chi 2$ analysis was used for categorical variables or Mann-Whitney U or independent two sample t tests were used for continuous variables. Univariate logistic regression analysis were applied to identify the risk factors on disease. Odds ratios were given with 95% confidence intervals. Analysis were conducted using Turcosa Cloud (Turcosa Ltd Co, Turkey) statistical softwares. A p value less than 5% was considered as statistically significant.

RESULTS

The sociodemographic statuses of the study groups are presented in Table 1-2. While there were differences in terms of occupation, mariatal status, education, family history of MS or another cerebral disease, vaccination, history of childhood diseases, poor bowel habits, consumption of fats-vegetables- yoghurt and presence of allergy between the groups, there were not difference between the groups in terms of age, gender, birthplace, dental fillings, water supply and place of roadside, and place of near to factory. The level of Ag, Al, As, Ba, Cd, Cs, Cu, Mn, Ni, Pb, Rb, Sr, Sia were higher in the patient groups compared to the control group. The level of Ti and Fe were lower in MS patients as compared to the control group in Table 3.

The results of univariate logistic regression analysis are given in Table 4. We observed that the high level of sialic acid was 14 times, heavy metals such as Ni, Cd, Al, As, Pb, Mn, Ag, Cs, Ba, Zn approximately 1.5 times more risky for MS. Additionally, we observed that the consumption of vegetable was 6.5 times less risky, bad bowel habits 6 times risky for MS.

DISCUSSION

It is known that MS is a disease affected by a diversity of genetic and environmental factors (Giacoppo et al., 2014). In this study, Sia and urine Al, Mn, Ni, Cu, As, Rb, Sr, Ag, Cd, Cs, Ba and Pb levels of MS patients were found to be higher than control group. Ti, and Fe values of MS patients were found to be lower than the control group. Also, it was found that high levels of sialic acid and heavy metals, less vegetable consumption and bad bowel habits were risk factors for multiple sclerosis.

In the medical literature, we did not find any studies including evaluations of both urine heavy metal and saliva Sia levels of MS patients.

There are studies showing that high Sia levels are seen in many diseases (Varki, 2008; Sillanaukee et al., 1999). Particularly in various inflammatory diseases, serum Sia

Table 2. Socio- demographic characteristics of controls and multiple sclerosis patients (continue)

Dental fillings	59.6	61.0	0.895
Brain disease such as MS in family	36.5	17.1	0.031
Childhood diseases	40.4	17.1	0.022
Vaccination			
Diphtheria-pertussis-tetanus	96.2	80.5	0.020
Measles rubella mumps	22.0	5.8	0.023
Smokers	38.5	26.8	0.050
Poor bowel habits	71.2	34.1	<0.001
Oil consumption		e	
Butter	21.2	-	
Margarine	-	2.5	<0.001
Oil	-	26.8	
Mix (margarine-oil)	78.8	70.7	
Oil type	4.9	43.9	
Olive oil	92.7	56.1	<0.001
Flower oil	2.4	-	
Corn oil			
Everyday vegetable consumers	26.9	70.7	<0.001
Everyday yogurt consumers	32.7	48.8	0.004
Kind of water			
Bottel water	17.3	29.3	0.363
Municipal water	78.8	65.9	
Ground water	3.9	4.8	
Place of roadside			
Yes	53.8	63.4	0.352
No	46.2	36.6	
Place of near to factory			
Yes	32.7	19.5	0.150
No	67.3	80.5	
Allergy	53.8	31.7	0.033

levels were shown to elevate in the acute phase of the disease and to decrease gradually in the course of treatment (Schauer 2009, Sillanaukee et al., 1999). In the absence of sialin, which is a Sia transporter, there were a decrease in myelin, in the myelin binding protein, which is the most important structural protein of myelin and a reduction in the number of the myelin producing mature oligodendrocytes in central nervous system (Prolo et al., 2009). In another study, when Sia is removed from the cell membrane, macrophages accept it as 'eat' signal (Varki et al., 2012). In this study, Sia values of MS patients were found higher than control. It is thought that Sia elevation may reflect an acute phase reaction giving information about the inflammation process.

In a study, it has been reported that membrane bound Sia and some metals were complexed and in another study, it was reported that metal toxicity caused an increase in Sia levels in tissue and plasma (Aktaç et al., 2010, Patel et al., 1993). Sia shows great affinity to toxic metals such as Cd and Pb (Saladini et al., 2002). In an epidemiological study, increased risk for neurodegenerative diseases such as MS is shown to be associated with environmental toxins such as heavy metals (Visconti et al., 2005). It is also known that the accumulation of Pb, Mo, As, Al plays a role in MS etiology (Tsai et al., 2013). It is shown that the metals can lead neurodegeneration by deterioration of the mitochondrial functions, which loss of ATP, the increase of reactive oxygen species and eventual leads cell death (Giacoppo et al., 2014; Tamburo et al., 2015, Alimonti et al., 2007, Haider 2015). There are also data showing that oxidative stress caused by metal accumulation leads to demyelination and axonal damage (Giacoppo et al., 2014, Tamburo et al., 2015). It has been reported that myelin sheath is susceptible to oxidative damage in particularly (Tamburo et al., 2015). Supporting these findings, in this study, metal and Sia levels of MS patients were found to be higher than the control group. We also think that this increase in Sia levels develops connected to the inflammation that is formed as a result of damage caused by metals.

However, all of the patients in this study were using interferon. There are studies showing that interferons decrease antigen presentation, leucocyte proliferation and T cell migration and that interferons create anti-inflammatory effect (Airas et al., 2007, Kasper et al., 2014). However, we did not find a study on the relationship between interferon and Sia in the literature. If Sia is an indicator of inflammation and interferon produces anti-inflammatory effect, we expect the levels of Sia to be higher in our patients.

Fe is a necessary metal in enzymatic reactions such as oxygen transportation, DNA synthesis, oxidative

Table 3. Levels of Heavy metals in urine samples and sialic acid in saliva samples of controls and multiple sclerosis patients

Heavy metals (µL)	**Controls (n=41)**			**MS patents (n=52)**			
	Median	**Percentiles**		**Median**	**Percentiles**		
		25	**75**		**25**	**75**	***p***
Aluminum (Al)	1.82	0.00	4.62	5.04	1.71	7.00	<0.001
Arsenic (As)	1.57	0.00	3.06	3.30	1.87	4.98	<0.001
Argentum (Ag)	0.00	0.00	3.07	2.18	1.25	5.46	<0.001
Barium (Ba)	4.06	0.00	5.95	5.02	2.55	6.55	0.025
Beryllium (Be)	1.68	0.00	6.46	2.34	1.98	2.95	0.073
Bismuth (Bi)	2.24	1.38	5.97	3.13	1.83	5.41	0.070
Calcium (Ca)	3.11	1.24	5.16	2.62	1.61	5.76	0.493
Cadmium (Cd)	0.00	0.00	0.00	0.00	0.00	1.13	0.002
Cobalt (Co)	4.14	1.62	5.95	4.38	3.17	6.22	0.179
Chromium (Cr)	2.38	1.25	6.20	2.01	1.39	5.38	0.612
Caesium (Cs)	1.04	0.00	2.64	2.16	1.42	3.20	<0.001
Copper (Cu)	0.00	0.00	5.11	2.61	0.00	4.60	0.044
Iron (Fe)	3.50	1.29	6.66	1.94	1.82	2.08	0.005
Mercury (Hg)	1.47	0.00	4.38	2.02	1.63	2.56	0.070
Lithium (Li)	2.87	1.30	7.21	3.02	1.78	4.28	0.966
Magnesium (Mg)	4.07	1.75	6.24	3.42	2.03	6.82	0.877
Manganese (Mn)	2.82	1.80	5.83	4.76	3.53	5.77	0.003
Sodium (Na)	2.41	1.35	6.62	2.93	2.50	4.91	0.426
Nickel (Ni)	1.62	0.00	3.48	3.79	1.67	5.22	<0.001
Lead (Pb)	2.50	0.53	5.62	5.20	3.88	6.58	0.001
Rubidiium (Rb)	1.79	1.22	4.43	3.20	1.92	5.05	0.017
Selenium (Se)	3.12	1.32	5.93	1.52	1.14	7.19	0.241
Stannum (Sn)	1.79	1.57	4.56	2.95	1.76	4.40	0.144
Strontium (Sr)	1.73	1.14	4.52	3.14	1.83	5.71	0.024
Titanium (Ti)	2.67	1.81	5.51	2.00	1.22	4.39	0.033
Uranium (U)	1.65	0.00	2.00	1.69	1.49	2.20	0.128
Vanadium (V)	2.78	1.48	4.80	3.34	2.54	4.64	0.061
Zinc (Zn)	4.17	1.19	5.69	4.74	2.00	6.97	0.060
Sialic acid	0.090	0.050	0.50	2.55	2.03	3.55	<0.001

Table 4. Univariate logistic regression analysis indicating the risk factors of MS patients

Variables	**Univariate Logistic Regression**		
	OR	**95 % Cl**	***p***
Sialic acid	14.23	4.83-41.89	<0.001
Everyday vegetable consumers			
Yes	1.00	-	-
No	6.56	2.64-16.30	<0.001
Bowel habits			
Good	1.00	-	
Poor	4.76	1.97-11.48	0.001
Ni	1.47	1.18-1.84	0.001
Cd	1.45	1.03-2.05	0.033
Al	1.39	1.17-1.66	<0.001
As	1.39	1.13-1.71	0.002
Pb	1.32	1.10-1.57	0.002
Mn	1.29	1.06-1.57	0.013
Ag	1.26	1.06-1.48	0.007
Cs	1.23	1.01-1.48	0.031
Ba	1.21	1.03-1.42	0.019
Zn	1.18	1.00-1.39	0.047
Fe	0.52	0.36-0.73	<0.001

OR: Odds Ratio,
CI: Confidence Interval

phosphorylation, synthesis of neurotransmitters and formation of myelin (Stankiewicz et al., 2014). There is a study revealing that iron protects the integrity of myelin and oligodendrocytes (Stephenson et al., 2014). Abnormalities in iron metabolism may lead to neuronal death and abnormal iron deposition in the MS brain (Varki et al., 2012, Visconti et al., 2005, Haider et al., 2015). It has been shown that as a result of accumulation of iron, oxidative cell damage and neurodegeneration is seen in the brain (Tamburo et al. ,2015, Haider et al., 2015, Stankiewicz et al., 2014, Stephenson et al., 2014, Exley et al., 2006). The latest studies supports the accumulation of iron in MS and it focuses on its role in pathogenesis (Tamburo et al., 2015, Haider et al., 2015). In two studies, it has been shown that peripheral iron levels are low in MS patients. They suggests that the presence of a mechanism in MS patients that either increases iron turnover or facilitates the dislocation of Fe from peripheral fluids into the brain (Visconti et al., 2005, Alimonti et al., 2007). Also in this study, urine iron levels of our MS patients have been found to be lower when compared to controls. However, this is study in which urine iron levels of MS patients have been found to be higher than those of the controls (Exley et al., 2006).

In this study, it was observed that the patient group consumed less vegetables than the control and had higher Sia levels. Vegetables are the main component of the diet because of having vitamins, minerals and other ingredients. Particularly the green leafy vegetables that are rich of phenolic compounds are reported to have biological functions including antioxidant and antimicrobial activities (Kim et al., 2013). Furthermore vegetables buffer the compounds of acid formed during digestion. Epidemiological studies show that consuming vegetables rich from natural antioxidants helps prevent chronic diseases such as cardiovascular diseases and cancer (Kim et al., 2013, Kaur et al., 2001). It is thought that this effect is due to the chelation of prooxidant metal ions (Kaur et al., 2001). However containing essential substances, vegetables may contain toxic elements. Vegetables get heavy metals mainly from irrigation water and from soil and via leaves that have been exposed to polluted air. Continuous consumption of these vegetables, even in low doses causes accumulation of metals in the body. (Islam et al., 2014).

In this study, it has been observed that MS patients consume less vegetables than the healthy control group. However all the metals except Fe have been determined to be higher in MS patients. Consuming more vegetables control group has low levels of Sia and heavy metals.

Recently, the gut microbiota has emerged as a potential factor in the development of MS and shown that patients with MS exhibit gut dysbiosis. The gut microbiota helps the host remain healthy by regulating various functions, including maintenance of the intestinal barrier, inhibition of colonization by pathogenic organisms, and shaping of both mucosal and systemic immune responses. Alteration of the gut microbiota may lead to intestinal and systemic disorders such as MS (Freedman et al., 2018).

We have observed that MS patients consumed less of yogurt, which is rich in probiotics, and fiber-rich vegetables than control group. Also, it was seen that 71.2 % of MS patients had poor bowel habits. In this case, we think that the bowel microbiate is not working properly.

As a result, we think that, high doses of heavy metals may be inhibit the function of intestinal microbiates. Additionally, heavy metals remove sia from the tissues by binding to it and it may make the tissues vulnerable to inflammatory attacks. On the other hand, intestinal permeability may increase as the microbiate cannot function properly. Thus, many microorganisms can pass into the system and cause many diseases such as MS.

Limitation of this study

The majority of the patients did not want to participate the study. Also another limitation was that the participants had some difficulties in remembering the past events.We could not determine CRP levels, heavy metals in drinking water and brain iron deposition.

ACKNOWLEDGEMENTS

This study was supported by the Erciyes University Scientific Research Project Unit with project code 5165.

Conflict of interest

There is no conflict of interest.

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