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Serum anti-measles IgG is affected by serum iron level in patients with beta thalassemia major

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Measles remains a significant cause of morbidity and mortality despite the availability of an effective vaccine. This study compared the impact of serum iron on the immunogenicity of the measles vaccine in healthy volunteers and beta thalassemia major patients. In this case-control study, 180 beta thalassemia major patients (cases) were compared to 180 healthy volunteers (controls). The study received ethical approval, and all participants provided informed consent. IgG antibody responses to measles were measured using the ELISA method, and the association between serum iron and serum anti-measles IgG concentrations was analyzed using linear regression. The results showed that immunity to measles was achieved in 98.3 % of controls and 93.9 % of cases volunteers, with a significant difference between the two groups (p=0.026). The mean serum IgG concentration was significantly higher in controls compared to cases (296.4 ± 60.0 and 254.9 ± 35.7 IU/mL, respectively, p<0.001). Among controls, immune individuals had a significantly higher serum IgG concentration than non-immune individuals (59.3 ± 27.5 and 21.0 ± 6.9 IU/mL, respectively, p=0.034), whereas immune patients had a significantly lower mean serum IgG concentration than non-immune patients (166.8 ± 33.1 and 247.9 ± 49.2 IU/mL, respectively, p<0.001). Linear regression analysis confirmed that serum IgG concentrations were directly related to serum iron levels in controls, but indirectly associated with serum iron levels in patients. In conclusion, our results suggest that both iron deficiency and iron overload are associated with lower serum anti-measles IgG antibody levels in controls and patients, respectively. Therefore, maintaining serum iron concentrations within the normal range is recommended for a better response to vaccination.

Keywords: measles; immunity; iron; beta thalassemia; healthy volunteers.

Introduction

Beta thalassemia syndromes are inherited blood disorders involving an abnormal form of hemoglobin, which leads to disturbances in beta globin chain. Active erythropoiesis and anemia are among the consequences of this disorder. Although beta thalassemia occurs through the world, it is most prevalent in North Africa, the Mediterranean area and Western Asia.[(1)] Annually, the incidence of beta thalassemia is estimated to be 1 in 100,000 in the world.[(2)] In Iran, is more prevalent in Northern and Southern regions of the country, where the carrier rate for α-thalassemia is around 35 % and for beta thalassemia is about 10 %.[(1)]

Thalassemia patients usually require regular blood transfusions and are at risk of iron overload (iron accumulation in the body as a result of red blood cell

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destruction). Iron overload is toxic for body tissues and organs especially for heart and liver.[3] Also, iron overload mainly causes immune abnormalities such as decrease in immune response and monocyte and macrophage function, that reduce immunity and enhance risk of infections.[4] Most patients with beta thalassemia may require splenectomy due to hypersplenism, symptomatic splenomegaly, or severe pancytopenia that may increase the risk of infectious diseases, since thalassemia is associated with a reduced specific antibody response.[2]

Measles is a very communicable viral infection that can lead to severe complications such as otitis media, thrombocytopenia, pneumonia and meningoencephalitis, especially in individuals with weakened immune systems.[5] Despite government efforts and the extensive immunization programs, measles results in more than 100,000 deaths annually,[5] particularly in low-income countries.[6]

The World Health Organization (WHO) has established the elimination of measles as a main public health concern. Therefore, it is essential that two doses of the measles-mumps-rubella (MMR) vaccine be provided to all individuals to eliminate the risk of infection.[7] Regularly in Iran, the MMR vaccine is administered to under 7 years old infants at 12 and 18 months after birth.[8] Administering the MMR vaccine to recipients of blood products represents a precaution. Therefore, a 3 - 6 month interval is recommended between vaccination and transfusion according to the transfused blood product.[8] In the other hand, iron overload creates an antigen exhibition blockage that may interfere with vaccine effect. An association of measles vaccination with an appropriate response has been shown in patients with transfusion-dependent diseases and healthy volunteers.[9] In Pakistan, only 23 % of thalassemia patients vaccinated against hepatitis B have protective antibodies.[10]

Understanding the association between serum iron levels and immunity to measles virus is fundamental in establishing effective vaccination strategies for this risk group. Therefore, the aim of the present work was to investigate the association of serum iron levels with immunity to measles vaccination in patients with beta thalassemia major and healthy volunteers.

Materials and Methods

A case-control study was carried out. One hundred and eighty patients with beta thalassemia major (92 splenectomized patients and 88 non-splenectomized patients) that were under the auspices of Jahrom Medical Sciences University were included in the study through census, as case group, and 180 healthy volunteers from the population of the same city who matched the patients in terms of age and gender who were without history of any disorders were selected as the control group.

Inclusion criteria: all patients in the thalassemia ward who regularly received blood were included in the study by census, and a control group of healthy individuals of the same age and sex were included in the study.

Exclusion criteria: individuals who had received immunosuppressive drugs in the past month, individuals who had undergone any immunotherapy in the past 7 months, and healthy individuals who had received any blood products in the past 3-8 months were excluded from the study.

The study was approved by the local ethical committee (ethical research number: IR.JUMS.REC.1401.154) and all participants were asked to complete an informed consent. Demographic data such as age, sex and history of splenectomy were recorded by a questionnaire. All participants received two doses of MMR vaccine at 12 and 18 months after birth according to Iranian vaccination program.

At the beginning, 5 mL of blood samples were collected from all participants and were assayed for serum concentrations of measles specific IgG antibodies, alanine transaminase (ALT), aspartate transaminase (AST), iron, and ferritin.

Serum concentrations of ALT, AST and iron were determined by spectrophotometry assay.

The IgG antibodies were determined by enzyme-linked immunosorbent assay (ELISA) method using a human commercial kit (IBL, Ref number 30114056) following the manufacturer's instructions. The results according to the reference values for IgG antibodies were classified

as immune (IgG > 220 IU/mL), suspicious (120-220 IU/mL) and non-immune (<120 IU/mL).

The levels of serum specific ferritin were determined by an enzyme immunoassay kit (human ferritin ELISA (Biovender, Cat. No. RCD012R). The normal serum level of iron and ferritin is 40–120 µg/dL and 27-375 ng/mL, respectively.

Categorical variables were analyzed through frequencies and percentage, while quantitative variables were presented as mean ± standard deviation. The independent student t-test and One-way ANOVA test were used to compare means and the chi-square test was used to compare the ratios. Also, the dependency of antibody titers to serum iron and ferritin was analyzed by linear regression test. SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for the analysis. A p-value less than 0.05 was considered statistically significant.

Results

A total of 360 persons participated, 180 beta thalassemia patients and 180 healthy volunteers (Table 1). The two groups were similar in terms of sex, age and serum concentration of AST enzyme (p> 0.05). However, the mean serum concentrations of ALT enzyme (p= 0.040), iron (p< 0.001), and ferritin (p< 0.001) among patients were significantly higher than in healthy volunteers. In other words, 96.7 % and 98.9 % of patients with beta thalassemia major had high levels of serum ferritin and iron, respectively, while 72.8 % of healthy individuals had normal levels of serum ferritin and iron. Beta thalassemia major patients significantly had lower levels of anti-measles IgG antibodies in serum compare to healthy volunteers (p< 0.001). Also, healthy individuals were more immune against measles virus than the case group (98.3 % and 93.9 %, respectively, p< 0.001).

Table 1. Comparison of study variables between healthy volunteers and beta thalassemia major patients.

Variables	Healthy volunteers (n = 180)	Patients (n = 180)	P value
Sex			
Male, n (%)	98 (54.4)	98 (54.4)	>0.999
Female, n (%)	82 (45.6)	82 (45.6)	
Serum ferritin			
Low level, n (%)	43 (23.9)	0 (0.0)	
Normal level, n (%)	131 (72.8)	6 (3.3)	< 0.001
High level, n (%)	6 (3.3)	174 (96.7)	
Serum iron			
Low level, n (%)	49 (27.2)	0 (0.0)	
Normal level, n (%)	131 (72.8)	2 (1.1)	< 0.001
High level, n (%)	0 (0.0)	178 (98.9)	
Measles immunity			
Non immune, n (%)	3 (1.7)	11 (6.1)	0.026
Immune, n (%)	177 (98.3)	169 (93.9)	
Liver enzyme, AST, IU/dL, means ± SD	25.2 ±10.9	27.9 ±22.8	0.164
Liver enzyme, ALT, IU/dL, means ± SD	24.5 ±10.6	28.3 ±22.1	0.040
Iron, µg/dL, means ± SD	58.7 ±27.7	171.8 ±39.3	< 0.001
Ferritin, ng/mL, means ± SD	107.3 ±183.1	964.5 ±537.7	< 0.001
Anti-measles IgG antibody, IU/mL, means ± SD	296.4 ±60.0	254.9 ±35.7	< 0.001
Age (year), means ± SD	13.1 ±1.8	13.0 ±1.9	0.884

SD: standard deviation

Table 2. Comparison of studied variables between three study groups.

Variables	Healthy volunteers	Patients		P value
		Non-splenectomy	Splenectomy	
Iron, µg/dL: mean (SD)	58.7 (27.7)	173.9 (35.4)	169.8 (42.8)	< 0.001
Ferritin, ng/mL: mean (SD)	107.3 (183.1)	1018.1 (525.7)	913.2 (546.9)	< 0.001
Anti-measles IgG antibody, IU/mL: mean (SD)	296.4 (60.0)	252.6 (31.0)	257.2 (39.7)	< 0.001
Measles immunity				
Non immune: (n, %)	3 (1.7)	6 (6.8)	5 (5.4)	0.083
Immune: (n, %)	177 (98.3)	82 (93.2)	87 (94.6)	

SD: standard deviation.

The patients were divided into two groups: splenectomy and non-splenectomy (Table 2). In both groups, serum levels of ferritin, iron and anti-measles IgG antibodies were similar (p> 0.05). Therefore, these patient groups had significantly higher serum ferritin and iron levels and lower serum anti-measles IgG antibodies compare to healthy individuals (p< 0.001). Regarding measles immunity, 98.3 %, 93.2 % and 94.6 % of healthy volunteers and patients with and without spleen were immune against measles virus, respectively (p= 0.083). Of the non-immune groups, 0.6 %, 6.8 % and 0.0 % of healthy volunteers and patients with and without spleen had doubtful protection, respectively.

As is shown in Table 3, non-immune healthy individuals had significantly lower levels of serum iron compared to the immune groups (p = 0.034). However, serum concentrations of iron and ferritin abnormally were higher than in immune patients (p < 0.001).

Three healthy volunteers had low abnormal serum iron levels and were non-immune to the measles, while 25.50 % of immune healthy individuals had serum iron levels <40 ng/mL.

The serum anti measles IgG antibody levels in healthy volunteers were directly related to serum ferritin (p= 0.005) and iron levels (p < 0.001). But in patients, the

Table 3. Serum ferritin and iron levels according to the immunity to measles.

Study group	Healthy volunteers		P	Beta thalassemia major		P
Anti-measles IgG antibody	Non immune	Immune		Non immune	Immune	
Iron, µg/dL: mean (SD)	21.0 (6.9)	59.3 (27.5)	0.034	247.9 (49.2)	166.8 (33.1)	< 0.001
Ferritin, ng/mL: mean (SD)	8.7 (2.7)	109.0 (184.2)	0.647	1766.2 (655.8)	912.3 (487.5)	< 0.001

SD: standard deviation.

Table 4. Serum ferritin and iron levels according to anti-measles IgG level.

Variables	Healthy volunteers		P	Beta thalassemia major		P
	B constant	B standardized		B constant	B standardized	
Serum ferritin, ng/mL	185.7	0.107	0.005	-	-	-
Serum iron, µg/dL		0.841	< 0.001	382.4	- 0.817	< 0.001

B Constant: the unstandardized coefficient, representing the amount of change in the anti-measles IgG antibody level for a one-unit increase in the independent variable (serum ferritin or serum iron) while holding other factors constant. B Standardized: represents the standardized coefficient, showing the strength of the association. -: not significant, thus the amounts are not written.

serum anti measles IgG antibody levels were inversely associated to serum iron when variables entered in linear regression analysis model (Table 4).

Discussion

Children with beta thalassemia are born worldwide every year. Patients dependent on blood transfusions as those with beta thalassemia major, have an increased risk of secondary infection due to numerous factors such as anemia, ineffective erythropoiesis, hemolysis, iron overload, splenectomy and iron chelation therapy. Furthermore, the proven immune depressive action of viral infections represents a certain risk of developing bacterial co-infections. At the moment, infections and their associated complications are the second most common cause of death in transfusion dependent thalassemia.[2]

According to WHO the elimination of measles must be one of the top public health priorities.[6] Outbreaks occur where unvaccinated children are present. In recent times, enormous measles outbreaks have been reported in different countries, causing many deaths. Improving measles vaccination coverage and reduction of related deaths are a universal priority; susceptible populations should be particularly protected.[11] In Iran, the goal of eliminating measles has been pursued for many years and, since March 2008, all children received MMR vaccine twice, at 12 and 18 months after birth.[8]

The lower frequency of immunity to measles in thalassemia patients compared to healthy children in the present study is consistent with previous studies that showed a lower immune response to vaccination in these patients compared to healthy individuals.[12,13]

Our results showed that a favorable level of immunity (more than 90 %) in beta-thalassemia patients and healthy children protected against measles virus after 5 to 14 years of MMR vaccination. In comparison to the present study, Casale et al. showed that the prevalence of immunity to measles in non-transfusion-dependent and transfusion-dependent patients 6 years after vaccination was 88 % and 78 %, respectively.[14] Other study showed a measles immunity rate of 68 % in transfusion-dependent patients.[15]

In Iran, 88.6 % children aged 30-54 months who received at least two doses of MMR vaccine were seropositive.[16] Consistent with our results, a study conducted by Davidkin et al, revealed 95 % persistent measles viruses-specific IgG for 15 years after the second MMR dose.[17]

Our results showed a significant association between serum levels of iron and immunity to measles. In healthy volunteers, the serum concentrations of iron were higher in immune than in non-immune volunteers. Conversely in beta thalassemia major patients, the serum iron levels were lower than in immune beta thalassemia major patients. These results were not different between splenectomized and non splenectomized patients. Forty-nine healthy volunteers (27.2 %) exhibited serum iron levels below the normal range, of which three (1.7 %) were not immune to measles, and the remaining were immune to measles. Furthermore, two of the beta thalassemia major patients under study exhibited serum iron levels within the normal range, and both were found to be immune to measles. However, 93.8 % of the beta thalassemia patients under study, who exhibited serum levels above normal, were immune to measles.

The regression analysis demonstrated that lower serum iron level in healthy volunteers was associated to lower serum anti measles IgG antibody. But in patient groups, higher serum iron level was associated to lower serum anti measles IgG antibody. This means that in healthy individuals, low serum iron level reduced the immune response to the measles vaccine, while in the patients under study, increased serum iron levels weakened the immune response to the measles vaccine.

Iron is a vital element for maintaining immune homeostasis and the proper functioning of immune system cells.[18] Both, iron deficiency[19] and iron overload[20] leads to weak prognoses in long-term diseases and increased vulnerability to infection. Also, in cohort study in Kenya demonstrated that iron deficiency was stronger predictor of decreased response to diphtheria, pertussis, measles and pneumococcal vaccines.[19] Furthermore, compared to infants that did not receive iron, those who received iron at time of vaccination had higher anti-measles IgG.[19] This result advocates that frequently iron receiving in children with

iron deficiency at time of measles vaccine improves the primary response to the vaccine.

The limitations of this study include the small sample size and the lack of measurement of serum levels of other micronutrients such as zinc, copper, and manganese and their effect on immunity to measles.

Conclusions

The results of the present study show that iron deficiency and iron overload have a negative effect on the relative immunity obtained from vaccination in healthy individuals and in beta thalassemia major patients, respectively. Therefore, compensating for iron deficiency in healthy individuals and taking steps to reduce iron overload in thalassemia patients dependent on frequent blood transfusions may be beneficial in improving the humoral immune system response to measles vaccine. Further studies with larger sample sizes are recommended in this regard, as well as to investigate the effect of serum iron on the immune response to other vaccines.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

Masihollah Shakeri: participated in drafting and writing the manuscript.

Amirhossein Kamran Jahromi: involved in manuscript drafting, serum sample collection, and data acquisition.

Vahid Rahmanian: vonceptualized and coordinated the study, authored the manuscript, and addressed all reviewer comments.

Karamatollah Rahmanian: vontributed to study design and conducted the statistical analyses.

Abdolreza Sotoodeh Jahromi: contributed to the study's conceptualization, design, laboratory procedures, and study management, authored the manuscript, and incorporated all reviewer feedback.

All authors have read and agreed to the published version of the manuscript.

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La inmunoglobulina G antisarampionosa sérica es afectada por los niveles de hierro sérico en pacientes con beta talasemia mayor

Resumen

El sarampión sigue siendo una causa importante de morbilidad y mortalidad a pesar de la disponibilidad de una vacuna eficaz. Este estudio comparó el impacto del hierro sérico en la inmunogenicidad de la vacuna contra el sarampión en voluntarios sanos y pacientes con beta talasemia mayor. En este estudio de casos y controles, se comparó a 180 pacientes con beta talasemia mayor (casos) con 180 voluntarios sanos (controles). El estudio recibió la aprobación ética y todos los participantes dieron su consentimiento informado. Se midieron las respuestas de anticuerpos IgG contra sarampión mediante el método ELISA, y se analizó la asociación entre las concentraciones séricas de hierro y las concentraciones séricas de IgG contra sarampión mediante regresión lineal. Los resultados mostraron que la inmunidad al sarampión se alcanzó en el 98,3 % de los controles y en el 93,9 % de los casos voluntarios, con una diferencia significativa entre los dos grupos ($p = 0,026$). La concentración sérica media de IgG fue significativamente mayor en los controles que en los casos (296,4 ± 60,0 y 254,9 ± 35,7 UI/mL, respectivamente, $p < 0,001$). Entre los controles, los individuos inmunes tenían una concentración sérica de IgG significativamente mayor que los individuos no inmunes (59,3 ± 27,5 y 21,0 ± 6,9 UI/mL, respectivamente, $p=0,034$), mientras que los pacientes inmunes tenían una concentración sérica media de IgG significativamente menor que los pacientes no inmunes (166,8 ± 33,1 y 247,9 ± 49,2 UI/mL, respectivamente, $p < 0,001$). El análisis de regresión lineal confirmó que las concentraciones séricas de IgG estaban directamente relacionadas con los niveles séricos de hierro en los controles, pero indirectamente asociadas con los niveles séricos de hierro en los pacientes. En conclusión, nuestros resultados sugieren que tanto la deficiencia como la sobrecarga de hierro se asocian a niveles más bajos de anticuerpos IgG antisarampión en suero en controles y pacientes, respectivamente. Por lo tanto, se recomienda mantener las concentraciones séricas de hierro dentro del rango normal para obtener una mejor respuesta a la vacunación.

Palabras clave: sarampión; inmunidad; hierro; beta talasemia; voluntarios sanos.

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