Could Heterozygous Beta Thalassemia Provide Protection Against Multiple Sclerosis?

Background: Heterozygous beta thalassemia (HBT) has been proposed to increase the risk of developing autoimmune disease. Our aim in this study was to examine the prevalence of HBT among multiple sclerosis (MS) patients.

Material/Methods: HBT frequency was investigated in our MS group (243 patients with MS). Hemoglobin electrophoresis (HE) was carried out if MS patients had a mean corpuscular volume of (MCV) <80 fL and a mean corpuscular hemoglobin level of (MCH) <27 pg/L according to a complete blood count (CBC). If MCV was lower than 80 fL, MCH was lower than 27 pg/L, and Hemoglobin A2 equal to or higher than 3.5%, a diagnosis of HBT was established. The frequency of patients with HBT in our MS patient group was statistically compared with the prevalence of HBT in the city of Istanbul, where our MS patients lived.

Results: The HBT prevalence was 0.823% (2 patients) in the MS patient group. The prevalence of HBT in Istanbul has been reported to be 4.5%. According to the z-test, the HBT prevalence in our MS patient group was significantly lower than that in Istanbul (Z=6.3611, two-sided p value <0.0001, 95% confidence interval of prevalence of HBT in our MS patient group: 0.000998–0.029413).

Conclusions: Contrary to our hypothesis at the outset of study, the reduced HBT prevalence in the MS group compared to HBT frequency in the city of Istanbul might indicate that HBT is protective against MS.

MeSH Keywords: beta-Thalassemia • Multiple Sclerosis • Platelet Aggregation

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Background

Heterozygous beta thalassemia minor (HBT) is a benign blood disorder caused by a hereditary reduction in beta globin synthesis, which often leads to mild anemia and is characterized by hypochromic microcytic erythrocyte indexes [1]. HBT is prevalent in many regions of the world, including the Mediterranean countries, the northern coast of Africa, the Middle East, Central Asia, Southeast Asia, the Far East, and South America [1]. The highest beta thalassemia carrier frequencies have been reported to be in Cyprus (14%), Sardinia (10.3%), and Southeast Asia [1]. Although beta thalassemia major (BTM), which is caused by deficient or completely lacking beta globin synthesis, is a very serious disease, it is widely understood that subjects with HBT do not typically experience significant problems [1,2]. However, some publications have reported that several diseases were more frequently detected in subjects with HBT than in individuals without HBT [2]. The risks of birth defects, gestational diabetes, type 2 diabetes mellitus, renal diseases, bronchial asthma, osteoporosis, fibromyalgia, and depression were increased in HBT patients [2,3]. Furthermore, HBT has been proposed to increase the risk for autoimmune disorders [2]. For example, the prevalence of HBT is significantly increased in rheumatoid arthritis patients; similarly, the incidence of rheumatoid arthritis is increased in HBT patients compared to the general population [2]. The prevalence of HBT in systemic lupus erythematosus (SLE) patients was lower than that in the general population, but SLE has been reported to exhibit a more serious course in subjects with HBT [4,5]. The causes of these associations remain unknown. Multiple sclerosis (MS) is an autoimmune inflammatory disease that is associated with myelin sheath damage in the central nervous system [6]. After trauma, MS is the second most common cause of disability in young adults [6]. MS affects an estimated 1 per 1000 individuals in the United States [7]. Except for 1 case report, our search of the literature did not identify any studies investigating the relationship between HBT and MS [8]. The present study investigated whether the prevalence of HBT is increased in patients with MS.

Material and Methods

Study design, study population, and establishment of the HBT and MS diagnosis

This study was designed as a cross-sectional prevalence study. Adult patients with MS who were treated in the MS polyclinic of the Neurology Department of Bezmialem Vakif University between 2000 and 2015 were included in this study. The hospital records of adult patients with MS were screened for complete blood counts (CBC). The following approach was applied to identify the patients with HBT in the MS patient group: if the mean corpuscular volume (MCV) was less than 80 fl and the mean corpuscular hemoglobin (MCH) level was less than 27 pg/cell in the MS patient’s CBC, which was obtained before MS treatment started, hemoglobin electrophoresis (HE) was performed. Patients displaying an MCV <80 fl, an MCH level <27 µg, and an HbA2 level ≥3.5% were considered to have HBT. MS diagnosis was established according to the revised McDonald criteria (2010) for all of the patients, and for the patients diagnosed with MS before 2010, the MS diagnosis was re-evaluated [9].

Laboratory tests

CBC analysis was carried out using a Sysmex XT 1800i apparatus (Roche 2011, Kobe, Japan). Hemoglobin electrophoresis was carried out using a Shimadzu 20-A apparatus (Shimadzu-2013, Kyoto, Japan) using the high-performance liquid chromatography (HPLC) method.

Statistics

Numerical variables are presented as means with standard deviations, and the nominal variables are presented as ratios. The 1-proportion z-test was carried out to compare the prevalence of HBT in our study population with that in Istanbul. A 2-tailed p value of <0.05 was considered to be statistically significant.

Ethics

The study was approved by the Bezmialem Vakif University Medical Faculty Ethics Committee, and all of the subjects recruited for the study provided written informed consent.

Results

There were 243 patients with MS: 171 participants were female and 72 participants were male (70.4% female, 29.6% male) in the MS group. The mean age was 37.4 ±11.5 years (age range 15–65 years).

There were 202 patients in our study population who had normal MCV and MCH, whereas 41 patients had low MCV and MCH. Hemoglobin electrophoresis (HE) was carried out on 41 patients. The results showed that only 2 patients who had abnormal HEs were compatible with HBT. The transferrin saturation rate and/or ferritin levels of 39 patients were low, which was considered to be iron-deficiency anemia (Table 1). Then, hemoglobin, MCV and MCH levels of those 39 patients were normalized by means of oral and/or intravenous iron treatment. The HE results of the 2 patients were as follows: Hb A2 4.98%, Hb F 0.224%, Hb A 85.86%, and Hb A2 4.22%, Hb F 0.201%, Hb A 85.62%. In the city of Istanbul, where the MS...
patients lived, the HBT prevalence is 4.5%, according to the data reported on the Turkish-language website of the Turkish Hematology Association [10]. On the other hand, according to a study by Gedikoğlu et al., Istanbul displays an HBT prevalence of 9.8% [11]. The HBT frequency in our MS population was 0.823%. Based on the data of the Turkish Hematology Association, the z-test revealed a significantly lower HBT prevalence in our MS group than among the population of Istanbul (z statistic=6.344, p<0.001, 95% confidence interval of HBT prevalence in our MS patients group: 0.000998–0.029413).

A post hoc power analysis of our study was carried out, with the power of the study being 95%.

Discussion

Contrary to our hypothesis at the outset of study, we found a reduced HBT prevalence in the MS group compared to the HBT prevalence in the city of Istanbul.

Two mechanisms have been proposed to explain the trend of an association between autoimmunity and HBT [2]. The beta globin gene is located at the p15.5 locus of chromosome 11, and certain genes that have been demonstrated to exert immuno-regulatory effects are located very close to this locus. This close gene linkage between the beta globin gene and these immuno-regulatory genes might predispose subjects with HBT to autoimmunity [2]. Hemorphin, a protein that suppresses inflammation and neutrophil migration, is primarily released from the beta globin chain of hemoglobin via proteolytic cleavage in vivo. Because beta globin synthesis is reduced in HBT, it was proposed that reduced hemorphin synthesis and/or expression might lead to autoimmunity [2]. The studies described above prompted us to examine whether the HBT prevalence was increased in MS. However, we unexpectedly found that the prevalence of HBT in the MS group was lower than that of Istanbul.

Subjects with HBT display a lower frequency of cardiac and cerebral ischemic events than controls in some studies, and this difference was supported by a meta-analysis [12]. The reason for this result is unknown; however, it has been proposed that, in patients with HBT, cerebral and cardiac ischemic events might occur less frequently because of lower levels of cholesterolemia, lower blood pressure, and less frequent hypertension, and lower viscosity of the blood [12].

In 1963, Hilgarter et al. observed signs of skin and mucosal hemorrhage in some patients with beta thalassemia major (BTM), despite the fact that liver parenchymal damage did not occur and clotting factors (factor I, factor II, factor V, factor VII, and factor IX) and prothrombin time was normal [13]. In 1977, Gruppo et al. observed that platelets responded to ADP, epinephrine, and collagen with reduced aggregation in patients with sickle cell anemia during vaso-occlusive crisis, and that the platelet factor 3 availability was also high. This was interpreted as “Platelets may give an in vitro reduced aggregation response since they were previously activated in vivo”. However, they detected that platelets responded to in vitro ADP, collagen, and epinephrine with decreased aggregation in the period during which patients with sickle cell anemia were also completely asymptomatic, and they could not explain it [14].

In an investigation carried out in 1978, Eldor et al., who took those studies into consideration, observed a reduced platelet aggregation response to collagen, ADP, adrenaline, and ristocetin in patients with BTM and HBT compared to controls. The patients in this study had signs of skin and mucosal hemorrhage, such as bruising easily, epistaxis, and menometrorrhagia [13]. In this study, platelet factor 3 availability, bleeding

| Table 1. Demographic data and prevalence of HBT in MS group and Istanbul City. |

| Variables                                      | Number | Proportion, % |
|-----------------------------------------------|--------|---------------|
| MS patients                                    | 243    | 100.0         |
| Female patients with MS                        | 171    | 70.4          |
| Male patients with MS                          | 72     | 29.6          |
| MS patients with low MCV and MCH               | 41     | 16.9          |
| MS patients with iron deficiency anemia        | 39     | 16.1          |
| MS patients with HBT in the study              | 2      | 0.8           |
| Prevalence of HBT in Istanbul City             |        | 4.5           |

| Variables                                      | Mean ±SD | Range |
|-----------------------------------------------|----------|-------|
| Age of MS patients, years                      | 37.4±11.5| 15–65 |
time, and clot retraction were normal in patients with BTM [13]. After the re-suspension of the platelets of patients with BTM in normal plasma, the reduced aggregation response did not resolve. Eldor et al. interpreted the results of their study as platelet dysfunction due to platelet membrane disorder [13].

Based on these studies, we hypothesized that hypoactive platelets could cause the reduced incidence of cerebral and cardiac ischemic events in patients with HBT, and we conducted a study to test this hypothesis. That study was accepted and is currently in the process of being published in the Journal of the Pakistan Medical Association. In that study, we found that the plasma levels of beta thromboglobulin and platelet factor 4, which are chemokines stored in alpha granules in individuals with HBT, were comparable to those of the controls, whereas the serum levels of the membrane glycoproteins soluble p-selectin (sPS) and soluble CD40 ligand (sCD40L) were lower than those in the controls. A lower level of sPS and sCD40L in patients with HBT compared to controls is consistent with the in vitro thrombocyte aggregation test previously reported among patients with HBT, and it could be explained by impaired platelet functions belonging to the platelet function markers of the platelet membrane group [13,15,16].

Reduced platelet functions in HBT patients may not be reflected uniformly in all classes of platelet function markers. In patients with BTM and HBT, a reduced platelet aggregation response to epinephrine, collagen, ADP, and ristocetin could be explained by a defect or impairment in the glycoprotein structure of receptors on platelet membranes [13,15,16]. An impairment of platelet function might be congenital or acquired in beta thalassemia. An acquired platelet function defect may result from oxidative stress. Increased oxidative stress desialylates platelet glycoproteins, disrupting its function and structure [17]. In patients with BTM, serum glycoprotein levels are lower and desialylated serum glycoprotein quantities are higher compared with the control group [18]. Oxidative stress increased in both BTM and HBT [18,19]. Increased oxidative stress may disrupt the structure of the membrane glycoproteins on platelets and cause reduced platelet functions. While sCD40L and sPS are platelet function markers from the membrane glycoproteins group, BT and PF4 are alpha granule chemokines [20].

The functions of platelets are not limited to hemostasis. Platelets are also involved in immunity and inflammation [21]. Activated platelets release many pro-inflammatory mediators that could propagate and trigger inflammation [21]. Platelets have been shown to be activated in MS patients [21]. The number of platelet aggregates and platelet-derived micro-particles, and the expression of p-selectin in the platelet membrane have been reported to be increased in MS patients [22].

In experimental autoimmune encephalomyelitis (EAE), an animal model of MS, platelet depletion substantially ameliorated autoimmune inflammation in the central nervous system [21]. Glatiramer acetate (GA) is a drug that has been approved by the FDA for the treatment of MS that very efficiently suppresses neuro-inflammation in EAE [21]. Although this drug was designed to inhibit pathogenic T cells, its complete mechanism of action is unknown [21]. A recent study showed that GA prolonged bleeding time and inhibited platelet activation in humans [21]. This result indicated that GA targets platelets, thereby inhibiting their interaction with immune cells [21]. Based on these observations, we think that impaired platelet functions in HBT patients might protect them from MS.

The CD40/CD40L system is expressed not only by platelets, but also by antigen-presenting cells (APCs), including macrophages, dendritic cells, and fibroblasts [23]. CD40 expression regulates the T cell-APC interaction and is centrally involved in inflammatory events. The CD40/CD40 L system was reported to be operational in MS [23]. Simultaneous immunization via proteolipid peptide injection to induce EAE and anti-CD40 monoclonal antibody administration to SJL mice effectively blocked EAE development [24]. EAE development was blocked even when the anti-CD40 monoclonal antibodies were administered several days after immunization [23]. However, once EAE developed, this intervention was ineffective during the late phases of this disease [24]. Based on the results of our previous study, the 2 platelet function markers belonging to the membrane glycoproteins group (P-selectin and sCD40L) were present at lower levels in individuals with HBT compared to controls, which might cause the lower prevalence of HBT in the MS population than in the general population.

The statistical power of our study is high. However, it is based on the comparison of HBT prevalence in the MS group with the prevalence of HBT in the city. A comparison of the incidence of MS in HBT patients with the incidence of MS in the general population is required for a definite conclusion. Our study is a preliminary study and may be seminal for future research.

Conclusions

The lower HBT prevalence in the MS population than that in the city of Istanbul, where the MS patients lived, suggests that HBT may be protective against MS. This may result from impaired platelet functions in HBT patients.

Statement

All of the authors state that there are no conflicts of interest.
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