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Original Article

Autosplenectomy in severity of sickle cell diseases

Mehmet Rami Helvaci, Can Acipayam, Ramazan Davran

Medical Faculty of The Mustafa Kemal University, Antakya

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Abstract: Background: We tried to understand whether or not there is an association between prevalence of autosplenectomy and severity of sickle cell diseases (SCDs). Methods: All SCDs patients with red blood cell (RBC) transfusions of less than 50 units in their lives were put into the first group and 50 units or higher were put into the second group. Results: The study included 316 patients (155 females). There were 224 cases (70.8%) in the first group and 92 cases (29.1%) in the second group ($p<0.001$). The male ratio was significantly higher in the second group (64.1% versus 45.5%, $p<0.001$). Although both the white blood cell and platelet counts were higher in the second group, there was a significant difference in platelet counts ($p=0.005$), and this was probably due to the small sample sizes. Although the prevalence of autosplenectomy was significantly higher in the first group (56.2% versus 45.6%, $p<0.05$), the mean number of painful crises per year, digital clubbing, chronic obstructive pulmonary disease (COPD), leg ulcers, stroke, chronic renal disease (CRD) and coronary heart disease (CHD) were significantly higher in the second groups ($p<0.05$ for all). Conclusion: In contrast to the lower prevalence of autosplenectomy, the mean number of painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, and CHD were significantly higher in the second group. So there may be an inverse relationship between prevalence of autosplenectomy and severity of SCDs, and spleen may act as a chronic inflammatory focus as a filter of blood for these abnormally hard RBCs.

Keywords: Autosplenectomy, sickle cell diseases, chronic capillary damage

Introduction

Probably systemic atherosclerosis is the main reason of aging by causing end-organ failures in human being. Although it mainly affects high blood pressure (BP) carrying arteries, the arterioles and capillaries are probably affected with some extent. Some of the triggering factors of the systemic process are overweight, elevated BP, dyslipidemia, and insulin resistance for the development of terminal end points such as obesity, hypertension, diabetes mellitus (DM), peripheric artery disease, osteoporosis, chronic obstructive pulmonary disease (COPD), chronic renal disease (CRD), cirrhosis, coronary heart disease (CHD), stroke, and aging, all of which are collected in the metabolic syndrome [1-6]. On the other hand, sickle cell diseases (SCDs) are systemic capillary processes affecting whole systems of body which are caused by homozygous inheritance of hemoglobin S (Hb S) [7, 8]. Hb S causes red blood cells (RBCs) to loss their normal elastic and biconcave disc shaped structures under oxidative stresses. Possibly loss of elasticity of the RBCs instead of their shapes is the major pathology of SCDs, since sickling is rare in the peripheric blood smears of patients with associated thalassemias, and the survival is not so affected in hereditary elliptocytosis or hereditary spherocytosis as in the SCDs. Probably loss of elasticity sustains during the whole life, and exaggerate with various stresses. The hard RBCs can take their normal elastic natures after normalization of the stressful conditions, but they getting hard with time. The hard cells induced chronic capillary damage, endothelial edema, and tissue ischemia, and finaly leading to infarcts. On the other hand, obvious vascular occlusions may not develop in greater vasculature due to transportation instead of their distribution functions for the hard RBCs. We tried to understand whether or not there is an association between prevalence of autosplenectomy and severity of SCDs in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University

Table 1. Sickle cell patients with the red blood cell transfusions

Variables	Cases with RBC* transfusions of less than 50 units	*p*-value	Cases with RBC transfusions of 50 units or higher
Prevalence	*70.8% (224)*	*<0.001*	*29.1% (92)*
Mean RBC units	*12.9 ± 11.2 (0-48)*	*<0.000*	*99.0 ± 56.5 (50-362)*
Mean age (year)	28.9 ± 9.9 (5-59)	ns†	30.0 ± 9.2 (9-56)
Male ratio	*45.5% (102)*	*<0.001*	*64.1% (59)*
Thalassemia minors	62.0% (139)	ns	58.6% (54)
Autosplenectomy	*56.2% (126)*	*<0.05*	*45.6% (42)*

*Red blood cell. †Nonsignificant ($p>0.05$).

between March 2007 and February 2014. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC). Their medical histories including numbers of painful crises per year, units of transfused RBC in their lives, smoking habit, regular alcohol consumption, leg ulcers, stroke, and surgical operations were collected. Cases with a history of one pack-year were accepted as smokers, and cases with a history of one drink a day for one year were accepted as drinkers. A check up procedure including serum iron, total iron binding capacity, serum ferritin, serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an electrocardiography, a Doppler echocardiography both to evaluate cardiac walls and valves, and to measure the systolic BP of pulmonary artery, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow in required cases, a computed tomography of brain, and a magnetic resonance imaging (MRI) of hips was performed. Other bone areas for avascular necrosis were scanned according to the patients' complaints. Cases with acute painful crises or any other inflammatory event were treated at first, and then the spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic BP of pulmonary artery, peripheral blood counts, renal and hepatic function tests, and measurement of serum ferritin were performed on the silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% [9]. Systolic BP of the pulmonary artery of 40 mmHg or higher during the silent phase is accepted as pulmonary hypertension [10]. A vascular necrosis of bones was detected via MRI [11]. Autosplenectomy is diagnosed ultrasonographically in the absence of any history of splenectomy. CRD is diagnosed with a permanently elevated serum creatinine level which is 1.3 mg/dL or higher in males and 1.2 mg/dL or higher in females on the silent phase. Cases with renal transplantation were put into the CRD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, ascites, and histologic procedure in case of requirement. Digital clubbing is diagnosed with the ratio of distal phalangeal diameter to interphalangeal diameter which is greater than 1.0, and with the presence of Swamroth sign [12, 13]. Associated thalassemia minors are detected by serum iron, total iron binding capacity, serum ferritin, and the hemoglobin electrophoresis performed via HPLC. A stress electrocardiography is performed in cases with an abnormal electrocardiography and/or angina pectoris. A coronary angiography is obtained just for the stress electrocardiography positive cases. So CHD was diagnosed either angiographically or with the Doppler echocardiographic findings as the movement disorders of the cardiac walls. Eventually, cases with RBC transfusions of less than 50 units in their lives were put into the first and 50 units or higher were put into the second groups, and the groups were compared in between. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 316 patients with SCDs (155 females and 161 males). There were 224 cases (70.8%) in the first and 92 cases (29.1%) in the second groups ($p<0.001$). There was a nonsignificant difference according to the prevalence of associated thalassemia minors between the groups (**Table 1**). Mean ages of the groups were similar, too (28.9 and 30.0 years, respectively, $p>0.05$). The mean units of transfused RBCs were 12.9 and 99.0, respectively ($p<0.000$). Interestingly, the male ratio

Table 2. Sickle cell patients with peripheric blood values

Variables	Cases with RBC* transfusions of less than 50 units	*p*-value	Cases with RBC transfusions of 50 units or higher
Mean WBC† counts (μL)	14.931 ± 6.791 (2.460-39.200)	ns‡	15.346 ± 5.640 (1.580-36.900)
Mean PLT§ counts (μL)	*435.670 ± 236.693 (48.000-1.827.000)*	*0.005*	*498.310 ± 224.570 (53.000-1.370.000)*
Mean hematocrit value (%)	23.8 ± 4.8 (11-42)	ns	23.7 ± 4.9 (13-39)

*Red blood cell. †White blood cell. ‡Nonsignificant ($p>0.05$). §Platelet.

Table 3. Clinical features of the sickle cell patients

Variables	Cases with RBC* transfusions of less than 50 units	*p*-value	Cases with RBC transfusions of 50 units or higher
Painful crises per year	*3.8 ± 6.3 (0-52)*	*0.000*	*8.4 ± 10.9 (0-52)*
Smoking	12.0% (27)	ns†	17.3% (16)
Digital clubbing	*7.1% (16)*	*<0.01*	*15.2% (14)*
Pulmonary hypertension	11.6% (26)	ns	10.8% (10)
COPD‡	*6.6% (15)*	*<0.001*	*20.6% (19)*
Leg ulcers	*11.6% (26)*	*<0.01*	*21.7% (20)*
Stroke	*5.8% (13)*	*<0.05*	*11.9% (11)*
CRD§	*4.9% (11)*	*<0.001*	*14.1% (13)*
Avascular necrosis of bones	20.5% (46)	ns	17.3% (16)
Cirrhosis	4.4% (10)	ns	4.3% (4)
CHD¶	*4.0% (9)*	*<0.05*	*8.6% (8)*
Rheumatic heart disease	8.4% (19)	ns	3.2% (3)
Exitus	4.4% (10)	ns	5.4% (5)

*Red blood cell. †Nonsignificant ($p>0.05$). ‡Chronic obstructive pulmonary disease. §Chronic renal disease. ¶Coronary heart disease.

was significantly higher in the second group (45.5% versus 64.1%, $p<0.001$). Although both the white blood cell (WBC) and platelet (PLT) counts of the peripheric blood were higher in the second group, the difference was only significant for the PLT counts ($p=0.005$), probably due to the small sample sizes of the study (**Table 2**). Mean hematocrit values were similar in the two groups (23.8% versus 23.7%, $p>0.05$). In contrast to the significantly lower prevalence of autosplenectomy, the mean number of painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, and CHD were significantly higher in the second group ($p<0.05$ for all) (**Table 3**). Mean ages of the mortal cases were 29.5 ± 9.8 (19-50) and 34.6 ± 6.7 (26-44) years in the first and second groups, respectively ($p>0.05$). Mean ages of the mortal cases were 29.7 ± 9.6 (19-50) and 33.3 ± 8.5 (21-44) years in males and females, respectively ($p>0.05$). On the other hand, there was no patient with regular alcohol consumption among the study cases. Although antiHCV was positive in two of the cirrhotics, HCV RNA was detected as negative by polymerase chain reaction in both.

Discussion

The SCDs mainly affect capillary endothelium [14], since the capillary system is the main distributor of the hard RBCs tissues. Due to the microvascular nature in microvascular complications of DM, we can observe complete healing of leg ulcers with hydroxyurea therapy in early years of life, but the healing process may be difficult due to the excessive fibrosis around the wounds later in life. Finally, the mean lifespan was 42 years in males and 48 years in females in the literature [15], whereas it was 29 and 33 years in males and females in the present study, respectively. The great differences may be secondary to the initiation of hydroxyurea therapy in early years of life in developed countries. On the other hand, the prolonged lifespan of females with SCDs and females in the world [16] can not only be explained by the atherosclerotic effects of smoking alone, instead it may be explained by

the dominant role of male sex in life [17]. Similarly, the male ratio was significantly higher in the second and severe group in the present study (64.1% versus 45.5%, $p<0.001$).

The spleen is found in all vertebrates with a similar structure to the lymph nodes. It acts primarily as a blood fitler, which removes old and abnormal RBCs and recycles the iron. Additionally, it synthesizes antibodies and removes antibody-coated bacteria and antibody-coated blood cells from circulation. Like the thymus, the spleen has only efferent lymphatic vessels, and it is the major lymphatic organ in the body. It has a central role in the reticuloendothelial system, and it retains the ability of producing lymphocytes after birth. The spleen acts as a pool of peripheral blood cells and release the cells in case of requirement. For example, it stores half of the body's monocytes in mice [18]. When injured, the monocytes can migrate to the injured tissue and transform into dendritic cells and macrophages which assist tissue healing [19]. It was detected in the present study that 56.2% cases of the first group and 45.6% of cases of the second group ($p<0.05$) had autosplenectomy, and these ratios were the highest ones compared with other affected tissues. So the spleen is probably the primarily affected organ in the body of such patients, and particularly due to the content of rich WBC, it may act as a chronic inflammatory focus. In the present study, a 28-year follow-up study of 740 veterans of World War II with surgical removal of spleen showed significant excesses of mortality caused by pneumonia and CHD [20], and the prevalence of CHD were significantly higher in the second group with lower prevalence of autosplenectomy.

Severe painful crises are nearly the pathognomonic symptoms of the SCDs, and they are precipitated by infection, operation, depression, or injuries. Although the painful crises may not be thought as a directly life threatening [21], increased basal metabolic rate associated with the crises may terminate with multiorgan failures on the chronic inflammatory background of the SCDs [22]. The severe pain is probably caused by the disseminated inflammation of the capillary endothelium, and the increased WBC and PLT counts and decreased hematocrit values may indicate presence of a chronic inflammatory process during whole their lives in such patients in the present study. Similar to our results, increased WBC counts even in silent periods was an independent predictor of the disease severity [23], and it was associated with an increased risk of stroke by causing disseminated capillary damage in brain [24]. Due to the severity of pain, narcotic analgesics are frequently required [25]. According to our practice, simple and repeated RBC transfusions are highly effective both to relieve pain and to prevent sudden death which may develop secondary to the multiorgan failures on the chronic inflammatory background. Simplicity of preparation of RBC suspensions in a short period of time provides advantages to clinicians to use them even in small public hospitals without the need of specialized health workers and equipments which are required for RBC exchange. Additionally, preparation of one or two units of RBC suspension makes it possiable for clinicians to prepare more units timely in case of sudden death. By this way, deaths during transportation to some extent can be prevented.

Hydroxyurea is a powerful drug for chronic myeloproliferative disorders and SCDs. It suppresses cell division by blocking formation of deoxyribonucleotides which hampered the synthesis of DNA. So hydroxyurea primarily acts on hyperproliferative cells. Although the action way of hydroxyurea is thought to be the increasing synthesis of gamma globin for fetal hemoglobin (Hb F) [26], its primary action may be the suppression of hyperproliferative WBC and PLTs in the SCDs. By this way, the continuous inflammatory process of the SCDs that initiated at birth on the capillary endothelium can be suppressed to some extent. Due to the same action way, hydroxyurea is also used to suppress hyperproliferative skin cells in psoriasis. Although a continuous damage of hard cells occurred in the capillary endothelium in the SCDs, the severity of destructive process is probably exaggerated by the patients' WBCs and PLTs as in autoimmune diseases. So the mechanism of -SCDs action in the body destructive process may mimic an autoimmune disease. The suppression of excessive proliferation of patients' WBC and PLTs by hydroxyurea may limit the capillary damage-induced tissue ischemia and infarcts all over the body. Similarly, lower neutrophil counts were associated with lower crises rates, and if a tissue infarction occurs, lower neutrophil counts may limit severity of pain and extent of tissue dam-

age [27]. On the other hand, after the use of hydroxyurea, final Hb F levels did not differ from their pretreatment levels significantly [27].

Hydroxyurea may play a vital role in the SCDs [14]. The Multicenter Study of Hydroxyurea studied 299 severely involved adults with sickle cell anemia (Hb SS), and compared the results of patients treated with hydroxyurea or placebo [28]. The study particularly searched effects of hydroxyurea on painful crises, acute chest syndrome, and requirement of RBC transfusions. The results were so overwhelming in the favour of hydroxyurea that the study was terminated after 22 months, and hydroxyurea was initiated to all patients. The patients treated with hydroxyurea had a 44% decrease of hospitalizations, and there was an independent association of lower neutrophil counts with the lower crisis rates [28]. But this study was performed on severe Hb SS cases alone, and the frequency of painful crises was decreased from 4.5 to 2.5 per year [28]. Whereas, we studied 337 patients with all subtype and severities of the SCDs, and the frequency of painful crises was decreased from 10.3 to 1.7 per year ($p<0.000$) with an additional decreased severity (7.8 versus 2.2, $p<0.000$) in a previous study [29]. Parallel to the above, adult SCDs patients using the drug appear to have reduced mortality rate after a 9-year follow-up [30]. Although the underlying disease severity remains vital to determine prognosis, hydroxyurea may decrease severity of disease [30] and prolong lifespan [14]. Chronic capillary damage is initiated at birth, and complications initiate to be seen in infancy. For instance, infants with lower hemoglobin levels, as an indicator of chronic inflammatory process, were more likely to have higher incidences of acute chest syndrome, painful crises, and lower neuropsychological scores, and hydroxyurea reduced the incidences of them [31]. Hydroxyurea initiated in early years of life may protect splenic function, improve growth, and prevent multiorgan dysfunctions by preventing chronic capillary damage. Transfusion programmes also reduce these complications, but they carry some significant risks including infections, development of alloantibodies, and iron overload. Additionally, using a drug orally is a much more easier method than regular transfusions for the patients, for their families, and for the health systems.

As a conclusion, in contrast to the lower prevalence of autosplenectomy, the mean number of painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, and CHD were significantly higher in the second group. So there may be an inverse relationship between prevalence of autosplenectomy and severity of SCDs, and spleen may act as a chronic inflammatory focus as a filter of blood for these abnormally hard RBCs in the SCDs cases.

Disclosure of conflict of interest

None.

Address correspondence to: Mehmet Rami Helvaci, Medical Faculty of The Mustafa Kemal University, 31100, Serinyol, Antakya, Hatay, Turkey. Tel: 00-90-326-2291000 (3333); Fax: 00-90-326-2455654; E-mail: mramihelvaci@hotmail.com

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