

Disappearance of hepatitis C virus antibodies with hydroxyurea therapy in sickle cell diseases

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Abstract

Background: We tried to understand whether or not there is a disappearance of hepatitis C virus antibodies (anti-HCV) with hydroxyurea therapy in patients with sickle cell diseases (SCD).

Methods: All patients with the SCD were included, and hydroxyurea therapy was initiated for all of them.

Results: The study included 337 patients (169 females and 168 males). Hydroxyurea therapy was well-tolerated with a high majority of cases (80.1%). Mean number of painful crises per year was decreased with the therapy (10.3 versus 1.7 crises per year, $p < 0.000$). Mean severity of painful crises was decreased, too (7.8 versus 2.2, $p < 0.001$). Although the body weight, mean hematocrit (Hct) value, and mean corpuscular volume (MCV) increased, white blood cells (WBC) and platelets (PLT) counts and the total and direct bilirubin, and lactate dehydrogenase (LDH) levels of plasma decreased with the therapy ($p < 0.000$ for all). Similarly, 23 patients (6.8%) with anti-HCV positivity before the therapy decreased to 16 patients (4.7%, $p > 0.05$) with hydroxyurea.

Conclusion: SCD are chronic inflammatory disorders with high morbidity and mortality rates, and hydroxyurea is a well-tolerated and highly effective regimen for them. While hydroxyurea therapy decreases both frequency and severity of painful crises, WBC and PLT counts, total and direct bilirubin, and LDH levels, it increases body weight, Hct value, and MCV. Although hydroxyurea therapy also decreased the anti-HCV positivity, the difference was nonsignificant probably due to the small sample size of the present study.

Key words: Sickle cell diseases, chronic endothelial damage, atherosclerosis, metabolic syndrome, hepatitis C virus antibodies, hydroxyurea

Introduction

Chronic endothelial damage may be the leading cause of aging and death. Probably whole afferent vasculature including capillaries are mainly involved in the process since much higher blood pressure (BP) of the afferent vessels may be the major underlying cause by inducing recurrent endothelial injuries. Thus the term of venosclerosis is not as famous as atherosclerosis in the literature. Secondary to the chronic endothelial damage, inflammation, edema, and fibrosis, arterial walls become thickened, their lumens are narrowed, and they lose their elastic nature, those reduce blood flow and increase systolic BP further. Some of the well-known accelerators of the atherosclerotic process are male gender, physical inactivity, excess weight, smoking, alcohol, and chronic inflammatory or infectious processes including sickle cell diseases (SCD), rheumatologic disorders, tuberculosis, and cancers for the development of irreversible consequences including obesity, hypertension (HT), diabetes mellitus (DM), peripheral artery disease (PAD), chronic obstructive pulmonary disease (COPD), chronic renal disease (CRD), coronary heart disease (CHD), cirrhosis, mesenteric ischemia, stroke, and benign prostatic hyperplasia (BPH) those terminate with early aging and premature death. They were researched under the title of metabolic syndrome in the literature, extensively (1-3). Although the early withdrawal of the causative factors may delay terminal consequences, the endothelial changes cannot be reversed after the development of obesity, HT, DM, PAD, COPD, CRD, CHD, stroke, or BPH due to their fibrotic nature (4-6). Similarly, SCD are systemic microangiopathic processes that are characterized by sickle-shaped red blood cells (RBC) caused by homozygous inheritance of the hemoglobin S (Hb S) (7, 8). Glutamic acid is replaced with a less polar amino acid, valine, in the sixth position of the beta chain of the Hb S. Presence of valine promotes polymerisation of the Hb S. So Hb S causes RBC to change their normal elastic and biconcave disc shaped structures to hard bodies. The decreased elasticity of RBC instead of their shapes may be the central pathology of the diseases. The sickling process is probably present in whole life, but it is exaggerated during various stressful conditions of the body. The RBC can take their normal elastic shapes after normalization of the stressful conditions, but after repeated cycles of sickling and unsickling, they become hard bodies, permanently. The hard cells induced chronic endothelial damage together with tissue ischemia and infarctions, even in the absence of obvious vascular occlusions, are the final consequences of the diseases, so life expectancy of such patients is decreased by 25 to 30 years (9). We tried to understand whether or not there is a disappearance of hepatitis C virus antibodies (anti-HCV) with hydroxyurea therapy in the SCD.

Material and Methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and September 2013. All patients with the SCD were enrolled into the study. SCD are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography. Their medical histories including frequency of painful crises per year and severity of them as a mean degree between 0 to 10 according to patient's self-explanation were detected. Cases with a history of three pack-year were accepted as smokers, and cases with a history of one drink a day for three years were accepted as drinkers. A check up procedure including body weight, 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, an abdominal ultrasonography, a computed tomography of brain, and a magnetic resonance imaging of hips was performed. Other bone areas for avascular necrosis were scanned according to the patients' complaints. Cases with acute painful crisis 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, and renal and hepatic function tests 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% (10). Systolic BP of the pulmonary artery of 40 mmHg or higher during the silent phase is accepted as pulmonary hypertension (11). CRD is diagnosed with a permanently elevated serum creatinine level of 1.3 mg/dL or higher on the silent phase. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, ascites, and liver biopsy in case of requirement. Digital clubbing is diagnosed with the ratio of distal phalangeal diameter to interphalangeal diameter of greater than 1.0 and with the presence of Schamroth's sign (12, 13). A stress electrocardiography was performed in cases with an abnormal electrocardiography and/or angina pectoris. A coronary angiography was 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. Then, the hydroxyurea therapy was initiated to all patients with an initial dose of 15 mg/kg/day, and then the dose was increased up to the final dose of 35 mg/kg/day according to patients' requirement and compliance. Finally, the mean number and severity of painful crises, body weight, white blood cells (WBC) and platelets (PLT) counts, hematocrit (Hct) value, mean corpuscular volume (MCV), and the total and direct bilirubin and lactate dehydrogenase (LDH) levels of the serum were compared before and after the hydroxyurea therapy. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 337 patients with the SCD (169 females and 168 males). Their mean ages were 28.4 ± 9.3 (8-59) versus 29.8 ± 9.3 (6-58) years in females and males, respectively ($p > 0.05$). The hydroxyurea treatment was used and well-tolerated with a high majority of cases (80.1%), and the remaining cases could not be followed up. We have not observed any major side effect of the therapy during the follow-up period. The final dose of 35 mg/kg/day was just achieved in 25 cases (7.4%), and the usual dose was 500 mg twice daily during the 7-year follow-up period. During the period, the mean number of painful crises per year was significantly decreased with the treatment (10.3 versus 1.7 crises per year, $p < 0.000$). The mean severity of painful crises was decreased, too (7.8 versus 2.2 , $p < 0.001$). Although the body weight, mean Hct value, and MCV increased, the WBC and PLT counts and the total and direct bilirubin and LDH levels of the serum decreased with the therapy, significantly

($p < 0.000$ for all). Parallel to the above developments, 23 patients (6.8%) with anti-HCV positivity before the therapy decreased to 16 patients (4.7%, $p > 0.05$) with hydroxyurea therapy (Table 1). On the other hand, we detected autosplenectomy in 46.8%, avascular necrosis of bones in 18.9% (90.6% at the hip joints), leg ulcers in 12.7%, pulmonary hypertension in 11.5%, CRD in 8.3%, CHD in 7.7%, digital clubbing in 6.5%, stroke in 6.5%, exitus in 5.3%, COPD in 4.7%, and cirrhosis in 3.2% of the patients (Table 2). Although smoking was observed in 6.5% (22) of the patients, there was only one case (0.2%) of regular alcohol consumption, who was not cirrhotic at the moment. Although anti-HCV was positive in two of the cirrhotics, HCV RNA was detected as negative by polymerase chain reaction in both. Prevalences of mortality were similar in both genders (4.7% versus 5.9% in females and males, respectively, $p > 0.05$), and mean ages of such cases were 32.1 versus 29.1 years in females and males, respectively ($p > 0.05$).

Table 1: Characteristic features of sickle cell patients before and after hydroxyurea therapy

Variables	Before hydroxyurea therapy	p-value	After hydroxyurea therapy
Mean number of painful crises per year	10.3 ± 10.6 (0-48)	<u><0.000</u>	1.7 ± 1.1 (0-6)
Mean severity of painful crises	7.8 ± 2.2 (0-10)	<u><0.000</u>	2.2 ± 1.7 (0-10)
Weight (kg)	59.1 ± 11.4 (37-95)	<u><0.000</u>	65.2 ± 13.0 (46-107)
WBC* (/ μ L)	15.050 ± 6.148 (4.890-38.800)	<u><0.000</u>	11.349 ± 5.029 (5.010-31.850)
Hct† value (%)	23.2 ± 4.0 (16-35)	<u><0.000</u>	27.8 ± 3.4 (20-36)
MCV‡ (fL)	88.7 ± 9.6 (57-112)	<u><0.000</u>	105.2 ± 13.6 (66-129)
PLT§ (/ μ L)	449.840 ± 217.370 (169.000-1.561.000)	<u><0.000</u>	430.840 ± 142.681 (219.000-936.000)
Total bilirubin value (mg/dL)	5.3 ± 5.6 (0.6-38.2)	<u><0.000</u>	3.1 ± 2.2 (0.7-11.0)
Direct bilirubin value (mg/dL)	2.0 ± 3.4 (0.2-15.0)	<u><0.000</u>	0.9 ± 0.9 (0.2-6.0)
LDH¶ value (IU/L)	647.5 ± 265.8 (196-1.552)	<u><0.000</u>	509.9 ± 315.4 (235-2.218)
Patients with anti-HCV** positivity	6.8% (23)	Ns***	4.7% (16)

*White blood cells †Hematocrit ‡Mean corpuscular volume §Platelets ¶Lactate dehydrogenase **Hepatitis C virus antibodies ***Nonsignificant ($p > 0.05$)

Table 2: Sickle cell patients with associated disorders

Variables	Prevalence
Autosplenectomy	46.8% (158)
Avascular necrosis of bones	18.9% (64)
Leg ulcers	12.7% (43)
Pulmonary hypertension	11.5% (39)
CRD*	8.3% (28)
CHD†	7.7% (26)
Digital clubbing	6.5% (22)
Stroke	6.5% (22)
Exitus	5.3% (18)
COPD‡	4.7% (16)
Cirrhosis	3.2% (11)

*Chronic renal disease †Coronary heart disease ‡Chronic obstructive pulmonary disease

Discussion

SCD particularly affect microvascular systems of the body (14, 15), since the capillary systems are the main distributors of the hard bodies to tissues, so they are destroyed much more than the larger vessels. Due to the prominent microvascular nature of the SCD, we can observe healing of leg ulcers with hydroxyurea therapy in early years of life, but later in life the healing process is difficult due to the excessive fibrosis around the capillaries. Eventually, the mean survival was 42 and 48 years for males and females in the literature, respectively (9), whereas it was 29.1 and 32.1 years, respectively, in the present study ($p>0.05$). The great differences between the survival should be searched with further studies, but it may be secondary to the delayed initiation of hydroxyurea therapy in Turkey. On the other hand, the prolonged survival of females with the SCD should also be searched, effectively. As a result of such a great variety of clinical presentation, it is not surprising to see that the mean body weight and body mass index (BMI) were significantly retarded in the SCD cases (16). Probably parallel to the significantly lower mean body weight and BMI, mean values of the low density lipoprotein cholesterol, alanine aminotransferase, and systolic BP and diastolic BP were also significantly lower in the SCD (16), which can be explained by definition of the metabolic syndrome (17, 18).

Painful crises are the most disabling symptoms of the SCD. Although some authors reported that painful crises themselves may not be life threatening directly (19), increased basal metabolic rate with any underlying cause including infections, tissue damage, operations, and depression usually terminate with painful crises and an increased risk of mortality. Probably pain is the result of a severe inflammatory process on the vascular endothelium all over the body, and the increased WBC and PLT counts and the decreased Hct values show presence of a chronic inflammatory process during whole their lives in such patients. For example, leukocytosis even in the absence of an infection was an independent predictor of the disease severity (20), and it was associated with an increased risk of stroke, probably by releasing cytotoxic enzymes and

causing endothelial damage (21). Due to the severity of pain, narcotic analgesics are usually required to control them (22), but according to our practice, simple, rapid, and repeated RBC transfusions are highly effective during the severe crises both to relieve pain and to prevent sudden death that may develop secondary to the multiorgan failures on chronic inflammatory background of the SCD.

Hydroxyurea is an effective therapeutic option for the treatment of chronic myeloproliferative disorders and SCD. It interferes with cell division by blocking the formation of deoxyribonucleotides via inhibition of ribonucleotide reductase. The deoxyribonucleotides are the building blocks of DNA. Hydroxyurea mainly affects hyperproliferating cells. Although the action way of hydroxyurea is thought to be the increase in gamma-globin synthesis for fetal hemoglobin (Hb F) (23, 24), we think that its main action way is the suppression of excessive leukocytosis and thrombocytosis via blocking the DNA synthesis in the SCD. By this way, the continuous inflammatory process of the SCD that initiated at birth on the vascular endothelium all over the body is suppressed with some extent. Due to the same action way, hydroxyurea is also used in moderate and severe psoriasis to suppress hyperproliferating skin cells. As in viral hepatitis cases, although presence of a continuous damage of sickled cells on the capillary endothelium, the severity of destructive process is probably exaggerated by the patients' own immune system particularly by the actions of WBC and PLT. So suppression of excessive proliferation of WBC and PLT probably limits the endothelial damage-induced tissue ischemia and infarctions all over the body. Similarly, it was reported that 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 damage (25). On the other hand, final Hb F levels in hydroxyurea users did not differ from their pretreatment levels, significantly (25).

Physicians at the National Institutes of Health Consensus Conference agreed that hydroxyurea is underused both in children and adults due to some reasons. Hydroxyurea is a chemotherapeutic agent, therefore it is not taken by

women planning to become pregnant in the near future. Additionally, there is a fear of potentially increased risk of cancers in people (26). However, the cancer risk has not been substantiated by more than a decade of using hydroxyurea for adults (27). Although the investigational and post-marketing data show risk to fetus (28), potential benefits may outweigh potential risk in pregnancy. According to our experiences, there are several female patients with infertility, abortus, or stillbirth in the absence of hydroxyurea therapy in the SCD, and the decreased number and severity of painful crises, increased body weight, decreased WBC and PLT counts, and increased Hct value will probably result with resolution of the above problems with some extent in such patients. It is clear that there is a need for more effective treatment regimens in the SCD, but until they become available, hydroxyurea should be used in all cases, and its dose should be kept as higher in the moderate and severe patients.

Hydroxyurea probably has a life-saving role in the SCD. As a similar result to our study, the Multicenter Study of Hydroxyurea (MSH) studied 299 severely affected adults with sickle cell anemia (Hb SS), and compared the results of patients treated with hydroxyurea or placebo (29). The study particularly searched effect of hydroxyurea on painful crises, acute chest syndrome (ACS), and requirement of blood transfusion. The outcomes were so overwhelming in the favour of hydroxyurea that the study was terminated after 22 months, and hydroxyurea was initiated in all patients. The MSH also demonstrated that patients treated with hydroxyurea had a 44% decrease in hospitalizations (29). In multivariable analyses, there was a strong and independent association of lower neutrophil counts with the lower crisis rates (29). But this study was performed just in severe Hb SS cases alone, and the rate of painful crises was decreased from 4.5 to 2.5 per year (29). Whereas in our study, we used all subtypes of the SCD with all clinical severity, and the rate of painful crises was decreased from 10.3 to 1.7 per year ($p < 0.000$) with an additional decreased severity of them (7.8 versus 2.2, $p < 0.000$). Parallel to our results, adult patients using hydroxyurea for frequent painful crises appear to have reduced mortality rate after a 9-year follow-up period (30). The underlying disease severity remains critical to determine prognosis, but hydroxyurea may decrease severity of disease (30). Probably the chronic endothelial damage of the SCD is initiated at birth, and complications may start to be seen even in infancy. For example, infants with lower hemoglobin levels were more likely to have a higher incidence of clinical events such as ACS, painful crises, and lower neuropsychological scores, and hydroxyurea reduced the incidence of them (31). Hydroxyurea therapy in the early years of life may also protect splenic function, improve growth, and prevent multiorgan dysfunctions by reversing early capillary damage. Transfusion programmes also reduce all of the complications of the SCD, however transfusions carry many risks including potential infection transmission, development of allo-antibodies causing subsequent transfusions more difficult, and iron overload.

HCV infection is an under-diagnosed and prevalent bloodborne illness, resulting in cirrhosis in up to 20% of

those infecteds (32). While the overall prevalence in the United States is 1.7%, high-risk populations may have up to a prevalence of 80% (32). About 170 million people are estimated to be chronically infected, worldwide (33). Additionally, it is the leading cause of hepatocellular carcinoma (HCC) and liver transplantation in developed countries (33). In addition to the hepatic involvement, it seems to be related with mixed cryoglobulinemia, Sjögren's syndrome (SS), rheumatoid arthritis (RA), B-cell non-Hodgkin's lymphoma (B-NHL), and membranoproliferative glomerulonephritis (MPGN) like many autoimmune disorders and malignancies (34). There are 36 reported extrahepatic, prominently autoimmune disorders which are thought to be related with HCV infection (35-40). For example, high prevalences of mixed cryoglobulinemia have been reported with chronic hepatitis C (41, 42). Its prevalence increases by the duration of infection, and the duration of infection is nearly two-fold longer in cases with mixed cryoglobulinemia (43). The prevalence of MPGN is approximately 30% in chronic hepatitis C plus type II cryoglobulinemia cases. In an autopsy study performed on 188 Japanese dominantly cirrhotic patients with chronic HCV infection, the prevalence of histological accumulation of immune complexes in glomeruli is importantly higher than the prevalence of symptomatic glomerulonephritis (GN) (39). The prevalence of histological GN was found as 54.8%, and the prevalence of MPGN as the most frequently seen type as 11.2%. But only 12.2% of cases, especially the MPGN having ones, were symptomatic for GN during the year just before death. According to a widely discussed hypothesis, the cause of extrahepatic involvement of HCV is the extrahepatic tropism, especially the lymphotropism of the virus. The lymphotropism is thought to be the important factor for the development of B-NHL and the production of autoantibodies. In long term follow up studies, B cell malignancies have been detected in 4-6% of cases with chronic hepatitis C plus type II cryoglobulinemia (44, 45). The prevalence of chronic hepatitis C has been found as higher in B-NHL cases than the controls in six studies performed in Italy and Japan (46-51). Although HCV is not accepted as an oncogenic virus until now, core proteins of HCV may take role in the malign conversion of cells (52). Additionally, oncogenesis and hypermutation of immunoglobulins of infected cells with HCV were shown (53). In addition to above, splenic lymphoma associating with chronic hepatitis C regresses parallel to the regression of viremia achieved by antiviral therapy (54). Additionally, although the detection of HCV specific CD4+ and CD8+ lymphocytes in lesions of lichen planus, they couldn't be detected in blood (55). The prevalence of HCV has been found as higher in patients with porphyria cutanea tarda in South Europa, America, and Japan. This finding supports the idea that HCV is a precipitating factor for porphyria cutanea tarda (56), since infection does not decrease the activity of hepatic uroporphyrinogen decarboxylase which is actually decreased in cases with active porphyria cutanea tarda (57).

HCV is believed not to be directly cytopathic, and the host immune response may be mainly responsible for the viral clearance and cellular injury. HCV persists in patients without any apparent evidence of immune deficits

depending on virus or host-related factors. The recent studies have revealed that both cellular and humoral immunity appear to be active despite the progression of the disease (58). Probably the genetically determined factors are also critical in eliminating hepatitis virus infections, and differences in host susceptibility to infectious disease and eventually the disease severity can't be attributed solely to the virulence of microbial agents. Immunologic factors such as human leukocyte antigens (HLA) may take role in the susceptibility to HCV (59). Class I HLA, which present foreign antigens to cytotoxic T-lymphocytes, are integral components of the early host immune response. Various major histocompatibility complex alleles that are correlated with more favorable outcomes in cases of viral hepatitis have been identified in diverse populations (60, 61). According to our experiences, HCV infection seems to be more frequent in elderlies. The increasing prevalence of HCV RNA positivity by age may indicate that it may eventually terminate with chronic manifestations in every infected if the life span of the individual permits. The anti-HCV persists for years after HCV infection, even in those individuals who present with HCV RNA negativity. Viremia can be intermittent in the first year of infection, and the presence of HCV RNA should be considered when attempting to determine the outcome of an acute HCV infection (62). The degree of liver damage can be semi-quantitatively assessed by a system used to score liver biopsies (63).

As a conclusion, SCD are chronic inflammatory disorders with high morbidity and mortality rates, and hydroxyurea therapy is a well-tolerated and highly effective regimen for them. While hydroxyurea therapy decreases frequency and severity of painful crises, WBC and PLT counts, total and direct bilirubin, and LDH levels, it increases body weight, Hct value, and MCV. Although hydroxyurea therapy also decreased the anti-HCV positivity, the difference was nonsignificant probably due to the small sample size of the present study.

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