Accelerated atherosclerosis and digital clubbing in sickle cell diseases

Mehmet Rami Helvaci (1)
Mustafa Sahan (2)
Agit Sulhan (1)
Abdullah Fatih Acik (1)
Adil Ocak (1)
Semih Salaz (1)
Lesley Pocock (3)

(1) Medical Faculty of Mustafa Kemal University, Department of Internal Medicine, M.D.
(2) Medical Faculty of the Mustafa Kemal University, Department of Emergency Medicine, M.D.
(3) medi+WORLD International

Correspondence:
Mehmet Rami Helvaci, M.D.
Medical Faculty of the Mustafa Kemal University,
31100, Serinyol, Antakya, Hatay, TURKEY
Phone: 00-90-326-2291000 (Internal 3399) Fax: 00-90-326-2455654
Email: mramihelvaci@hotmail.com

Abstract

Background: Sickle cell diseases (SCDs) are chronic destructive processes mainly on the capillary endothelium. We tried to understand the significance of digital clubbing in severity of SCDs.

Methods: All patients with SCDs were taken into the study.

Results: The study included 397 patients (193 females). There were 36 cases (9.0%) with digital clubbing. Male ratio was significantly higher in the digital clubbing group (66.6% versus 49.8%, $p<0.05$). The mean age was significantly higher in the digital clubbing group, too (36.5 versus 29.0 years, $p=0.000$). Additionally, smoking was also higher in the digital clubbing group, significantly (30.5% versus 11.0%, $p<0.001$). The mean white blood cell counts of peripheric blood were similar in both groups ($p<0.05$). On the other hand, the mean hematocrit value and platelet count of peripheric blood were lower in the digital clubbing group, significantly ($p= 0.001$ and $p= 0.012$, respectively). Beside that, prevalence of leg ulcers, pulmonary hypertension, chronic obstructive pulmonary disease, coronary heart disease, cirrhosis, and stroke were significantly higher in the digital clubbing group ($p<0.01$ for all). There were 25 mortalities during the period, and 13 of them were males. The mean ages of mortality were 33.0 ± 9.6 (range 19-47) in females and 30.0 ± 8.6 years (range 19-50) in males.

Conclusion: SCDs are chronic destructive processes on endothelium particularly at the capillary level, and terminate with accelerated atherosclerosis induced end-organ failures in early years of life. Probably digital clubbing is one of the terminal consequences of the SCDs indicating significantly shortened survival in such patients.

Key words: Sickle cell diseases, digital clubbing, chronic capillary damage, accelerated atherosclerosis, metabolic syndrome

Introduction

Chronic endothelial damage induced atherosclerosis may be the major cause of aging by inducing prolonged cellular hypoxia all over the body. For example, cardiac cirrhosis develops due to the disseminated hepatic hypoxia in patients with pulmonary and/or cardiac diseases. Probably whole afferent vasculature including capillaries are involved in the process. Some of the currently known accelerator factors of the destructive process are physical inactivity, overweight and smoking, for the development of irreversible consequences including obesity, hypertension, diabetes mellitus (DM), peripheric artery disease (PAD), chronic obstructive pulmonary disease (COPD), chronic renal disease (CRD), coronary heart disease (CHD), cirrhosis, mesenteric ischemia, osteoporosis and stroke, all of which terminate with early aging and death. They were researched under the title of metabolic syndrome in the literature, extensively (1-5). Similarly, sickle cell diseases (SCDs) are chronic destructive processes on endothelium mainly at the capillary level. Hemoglobin S (HbS) causes loss of elastic and biconcave disc shaped structures of red blood cells (RBCs). Probably, loss of elasticity instead of shapes of RBCs is the major problem, since sickling is very rare in the peripheric blood sample of the SCDs patients with associated thalassemia minors, and human survival is not so affected in hereditary elliptocytosis or spherocytosis. Loss of elasticity is probably present in the whole lifespan, but it is exaggerated with increased metabolic rate of the body. The hard cells induced prolonged endothelial inflammation, edema, remodeling, and fibrosis mainly at the capillary level and terminate with disseminated tissue infarcts all over the body (6,7). On the other hand, obvious vascular occlusions may not develop in greater vasculature due to the transport instead of distribution function of them. We tried to understand significance of digital clubbing in severity of SCDs in the present study.

Materials and Methods

The study was performed in the Medical Faculty of the Mustafa Kemal University between March 2007 and March 2015. All patients with SCDs were taken into the study. The SCDs are diagnosed with hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC) method. Medical histories including smoking habit, regular alcohol consumption, painful crises per year, surgical operations, priapism, leg ulcers, and stroke were learnt. Patients with a history of one pack-year were accepted as smokers, and one drink-year were accepted as drinkers. A check up procedure including serum iron, iron binding capacity, ferritin, creatinine, liver function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, a posterior-anterior chest x-ray film, an electrocardiogram, a Doppler echocardiogram both to evaluate cardiac walls and valves and to measure the systolic blood pressure (BP) of pulmonary artery, an abdominal ultrasonography, a computed tomography of brain, and a magnetic resonance imaging (MRI) of hips, was performed. Other bones for avascular necrosis were scanned according to the patients' complaints. So avascular necrosis of bones was diagnosed by means of MRI (8). Cases with acute painful crises or any other inflammatory event were treated at first, and then the laboratory tests and clinical measurements were performed on the silent phase. Stroke is diagnosed by the computed tomography of brain. Acute chest syndrome (ACS) is diagnosed clinically with the presence of new infiltrates on chest x-ray film, fever, cough, sputum production, dyspnea, or hypoxia in the patients (9). An x-ray film of abdomen in upright position was taken just in patients with abdominal distention and discomfort, vomiting, obstipation, and lack of bowel movement. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in one second/forced vital capacity of less than 70% (10). Systolic BP of the pulmonary artery of 40 mmHg or higher during the silent period is accepted as pulmonary hypertension (11). CRD is diagnosed with a serum creatinine level of 1.3 mg/dL or higher in males and 1.2 mg/dL or higher in females during the silent period. Cirrhosis is diagnosed with liver function tests, ultrasonographic findings, and histologic procedure in case of indication. 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 Schamroth's sign (12,13). Associated thalassemia minors are detected with serum iron, iron binding capacity, ferritin, and hemoglobin electrophoresis performed via HPLC method. Stress electrocardiography is just performed in cases with an abnormal electrocardiogram and/or angina pectoris. Coronary angiography is taken just for the stress electrocardiography positive cases. So CHD was diagnosed either angiographically or with the Doppler echocardiographic findings as the movement disorders in the cardiac walls. Rheumatic heart disease is diagnosed with the echocardiographic findings, too. Ileus is diagnosed with gaseous distention of isolated segments of bowel, vomiting, obstipation, cramps, and with the absence of peristaltic activity of the abdomen. Eventually, cases with digital clubbing and without were collected into the two groups, and they 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 397 patients with the SCDs (193 females and 204 males). There were 36 cases (9.0%) with digital clubbing. Mean age of patients was significantly higher in the digital clubbing group (36.5 versus 29.0 years, $p=0.000$). The male ratio was significantly higher in the clubbing group, too (66.6% versus 49.8%, $p<0.05$). Parallel to the male ratio, smoking was also higher in the digital clubbing group, significantly (30.5% versus 11.0%, $p<0.001$). Prevalence of associated thalassemia minors were similar in both groups (58.3% versus 66.2% in the clubbing group and other, respectively, $p>0.05$) (Table 1). The mean white blood cell (WBC) counts of the peripheric blood were similar in both groups ($p<0.05$). The mean hematocrit (Hct) value and platelet (PLT) count of peripheric blood were lower in the digital clubbing group, significantly

Table 1: Characteristic features of the study cases

Variables	Cases with digital clubbing	*p*-value	Cases without digital clubbing
Prevalence	9.0% (36)		90.9% (361)
Male ratio	***66.6% (24)***	***<0.05***	***49.8% (180)***
Mean age (year)	***36.5 ± 10.9 (16-56)***	***0.000***	***29.0 ± 9.7 (5-59)***
Thalassemia minors	58.3% (21)	Ns*	66.2% (239)
Smoking	***30.5% (11)***	***<0.001***	***11.0% (40)***

*Nonsignificant (p>0.05)

Table 2: Peripheric blood values of the study cases

Variables	Cases with digital clubbing	*p*-value	Cases without digital clubbing
Mean WBC* counts (/µL)	15.329 ±4.801 (7.000-26.600)	Ns†	15.114 ±6.756 (1.580-48.500)
Mean Hct‡ values (%)	***21.0 ± 4.3 (12-32)***	***0.001***	***23.9 ± 5.1 (8-42)***
Mean PLT§ counts (/µL)	***378.916 ± 184.460 (114.000-1.142.000)***	***0.012***	***461.116 ± 231.611 (48.800-1.827.000)***

*White blood cell †Nonsignificant (p>0.05) ‡Hematocrit §Platelet

Table 3: Associated pathologies of the study cases

Variables	Cases with digital clubbing	*p*-value	Cases without digital clubbing
Painful crises per year	5.0 ± 9.1 (0-36)	Ns*	5.2 ± 8.1 (0-52)
Tonsilectomy	2.7% (1)	Ns	8.0% (29)
Priapism	2.7% (1)	Ns	2.7% (10)
Ileus	8.3% (3)	Ns	3.3% (12)
Leg ulcers	***33.3% (12)***	***<0.001***	***11.9% (43)***
Pulmonary hypertension	***27.7% (10)***	***<0.001***	***9.6% (35)***
COPD†	***38.8% (14)***	***<0.001***	***12.1% (44)***
CHD‡	***27.7% (10)***	***<0.01***	***12.1% (44)***
CRD§	11.1% (4)	Ns	7.2% (26)
Rheumatic heart disease	5.5% (2)	Ns	6.0% (22)
Avascular necrosis of bones	13.8% (5)	Ns	22.9% (83)
Cirrhosis	***25.0% (9)***	***<0.001***	***1.6% (6)***
ACS¶	8.3% (3)	Ns	3.3% (12)
Stroke	***27.7% (10)***	***<0.001***	***6.9% (25)***
Mortality	8.3% (3)	Ns	6.0% (22)

*Nonsignificant (p>0.05)
†Chronic obstructive pulmonary disease
‡Coronary heart disease
§Chronic renal disease Acute chest syndrome

(p= 0.001 and p= 0.012, respectively) (Table 2). On the other hand, the prevalence of leg ulcers, pulmonary hypertension, COPD, CHD, cirrhosis, and stroke were significantly higher in the clubbing group (p<0.01 for all) (Table 3). Beside that there were 25 mortalities during the eight-year follow up period, and 13 of them were males. The mean ages of mortality were 33.0 ± 9.6 (range 19-47) in females and 30.0 ± 8.6 years (range 19-50) in males (p>0.05). Additionally, there were five patients with regular alcohol consumption who are not cirrhotic at the moment. Although antiHCV was positive in eight of the cirrhotics, HCV RNA was detected as positive just in two, by polymerase chain reaction method.

Discussion

Chronic endothelial damage induced atherosclerosis is the most common type of vasculitis, and it is the leading cause of morbidity and mortality in the elderly. Probably whole afferent vasculature including capillaries are involved in the body. Much higher BP of the afferent vasculature may be the major underlying cause, and efferent vessels are probably protected due to the much lower BP in them. Secondary to the prolonged endothelial damage and fibrosis, vascular walls become thickened, their lumens are narrowed, and they lose their elastic natures that can reduce the blood flow and increase BP further. Although early withdrawal of the causative factors including smoking, physical inactivity, excess weight, increased serum glucose and lipids, and elevated arterial BP may prevent terminal consequences, after development of COPD, cirrhosis, CRD, CHD, PAD, or stroke, the endothelial changes may not be reversed completely due to the fibrotic nature of them (14).

SCDs are life-threatening genetic disorders affecting nearly 100,000 individuals in the United States (15). As a difference from other causes of atherosclerosis, the SCDs probably keep vascular endothelium mainly at the capillary level (16), since the capillary system is the main distributor of the hard RBCs to tissues. The hard RBCs induced chronic endothelial damage, inflammation, edema, and fibrosis mainly at the capillary level and build up an advanced atherosclerosis in much younger ages of the patients. In other words, SCDs are mainly chronic inflammatory disorders, and probably the main problem is endothelial damage, inflammation, edema, and fibrosis induced occlusions in the vascular walls rather than the vascular lumens all over the body. As a result, the lifespans of patients with the SCDs were 48 years in females and 42 years in males in the literature (17), whereas they were 33.0 and 30.0 years in the present study, respectively. The great differences may be secondary to delayed initiation of hydroxyurea therapy and inadequate RBC transfusions in emergencies in our country. On the other hand, longer lifespan of females with the SCDs and longer overall survival of females in the world cannot be explained by the atherosclerotic effects of smoking alone, instead it may be explained by more physical power requiring role of male sex in life (18,19), since physical power induced increased metabolic rate may terminate with an exaggerated sickling and atherosclerosis in human body.

Digital clubbing is probably an indicator of disseminated atherosclerosis even at the capillary level, and it is characterized by bulbous enlargement of distal phalanges because of the increased soft tissue. Digital clubbing develops in the following steps; fluctuation and softening of the nailbed, loss of normal <165° angle between the nailbed and fold, increased convexity of the nail fold, thickening of the whole distal finger, and shiny aspect and striation of the nail and skin (20). Schamroth's window test is a well-known test for the diagnosis of clubbing (13). When the distal phalanges of corresponding fingers of opposite hands are directly opposed, a diamond-shaped 'window' is normally apparent between the nailbeds. If this window is obliterated, the test is positive. Digital clubbing is seen with pulmonary, cardiac, and hepatic disorders that are featuring with chronic tissue hypoxia (12,14), since lungs, heart, and liver are closely related organs that affect their functions in a short period of time. Similarly, hematologic disorders that are featuring with chronic tissue hypoxia may also terminate with digital clubbing. For example, we observed digital clubbing in 9.0% of patients with the SCDs in the present study and leg ulcers, pulmonary hypertension, COPD, CHD, cirrhosis, and stroke like other atherosclerotic disorders were significantly higher among them (p<0.01 for all). Similar to some other studies, there was a male predominance in the digital clubbing cases (66.6% versus 49.8%, p<0.05) that may also indicate roles of smoking on digital clubbing (12,14).

Smoking has a major role in systemic atherosclerotic processes such as COPD, cirrhosis, CRD, PAD, CHD, stroke, and cancers, too (21,22). Its atherosclerotic effects are the most obvious in Buerger's disease and COPD. Buerger's disease is an inflammatory process terminating with obliterative changes in small and medium-sized vessels and capillaries, and it has never been reported without smoking. COPD may also be a capillary endothelial inflammation terminating with disseminated pulmonary destruction, and it may be accepted as Buerger's disease of the lungs. Although it has strong atherosclerotic effects, smoking in human beings and nicotine administration in animals may be associated with weight loss (23). There may be an increased energy expenditure during smoking (24), and nicotine may decrease caloric intake in a dose-related manner after cessation of smoking (25). Nicotine may lengthen inter-meal time, and decrease amount of meal eaten in animals (26). Body weight seems to be the highest in former, lowest in current, and medium in never smokers (27). Since smoking may also show the weakness of volition to control eating, prevalences of HT, DM, and smoking were the highest in the highest triglyceride having group as a significant indicator of metabolic syndrome (28). Additionally, although CHD were detected with a similar prevalence in both sexes (22), smoking and COPD were higher in males against the higher prevalences of body mass index and its consequences including dyslipidemia, HT, and DM, in females.

COPD is an inflammatory disease that may mainly affect the pulmonary vasculature, and aging, smoking, and excess weight may be major causes of the inflammation. The inflammatory process of endothelium is enhanced by

release of various chemical factors by lymphocytes, and it terminates with fibrosis and atherosclerosis. Probably the accelerated atherscerotic process is the main structural background of the functional changes characteristic of the disease. Although COPD may mainly be an accelerated atherosclerotic process of the pulmonary vasculature, there are several reports about existence of an associated endothelial inflammation all over the body (29-30). For example, there may be a close relationship between COPD and CHD, PAD, and stroke (31). In a multi-center study performed on 5.887 smokers aged between 35 and 60 years, two-thirds of mortality cases were caused by cardiovascular diseases and lung cancers, and CHD was the most common cardiovascular complication among them (32). When the hospitalizations were searched, the most common causes were the cardiovascular diseases again (32). In another study, 27% of all mortality cases were due to the cardiovascular causes in the moderate and severe COPD patients (33). Similarly, beside digital clubbing, pulmonary hypertension, leg ulcers, and stroke, COPD may be one of the final consequences of the SCDs (34).

Leg ulcers are seen in 10 to 20% of patients with the SCDs (35), and the ratio was 13.8% in the present study. The incidence increases with age and they are rare under the age of 10 years (35). Leg ulcers are also more common in males and sickle cell anemia (HbSS) cases (35). They have an intractable nature, and around 97% of healed ulcers return in less than one year (36). The ulcers occur in distal areas with less collateral blood flow in the body (36). They are mostly seen just above the medial malleolus. The lateral malleoli are involved, secondly. Venous insufficiency is not a primary cause, but chronic endothelial damage at the microcirculation of the skin due to the hard RBCs may be the major cause in the SCDs (35). Prolonged exposure to the causative factors due to the blood pooling in the lower extremities by the effect of gravity may also explain the leg but not arm ulcers in the SCDs. Probably the same mechanism is also significant for the diabetic ulcers, Buerger's disease, and varicose veins. Smoking may also have an additional role for the ulcers (37), since both of them are more common in males (35), and atherosclerotic effects of smoking are well-known in COPD, CHD, PAD, and Buerger's disease (21,22).

Probably cirrhosis is also a systemic atherosclerotic process prominently affecting the hepatic vasculature, and aging, smoking, regular alcohol consumption, local and systemic inflammatory or infectious processes, excess weight, elevated BP, dyslipidemia, hyperglycemia, and insulin resistance may be the major causes of inflammation (38). The inflammation is enhanced by the release of various chemical factors by lymphocytes to repair the damaged endothelium of hepatic vasculature (39), and the chronic inflammatory process terminates with an advanced atherosclerosis and tissue hypoxia and infarcts. Although cirrhosis is mainly an accelerated atherosclerotic process of the hepatic vasculature, there may be a close relationship between cirrhosis and CHD, COPD, PAD, CRD, and stroke probably due to the underlying systemic atherosclerotic process (40). For example, most of the mortality cases in cirrhosis may actually be caused by cardiovascular diseases, and CHD may be the most common one among them (41). Similarly, beside digital clubbing, pulmonary hypertension, leg ulcers, stroke, and COPD like other atherosclerotic end-points, cirrhosis may be one of the final consequences of the SCDs (42).

Stroke is an important cause of death, and thromboembolism in the background of atherosclerosis is the most common cause of it. Aging, male sex, smoking, increased serum glucose and lipids, elevated arterial BP, and excess weight may be the major accelerator factors of it. Stroke is also a common complication of the SCDs (43,44). Similar to the leg ulcers, stroke is higher in HbSS cases (45). Additionally, a higher WBC count is associated with a higher incidence of stroke (46). Sickling induced endothelial injury, activations of WBC, PLT, and coagulation system, and hemolysis may terminate with chronic endothelial inflammation, edema, remodeling, and fibrosis (47). Probably, stroke is a complex and terminal event in the SCDs, and it may not have a macrovascular origin, instead disseminated capillary inflammation induced endothelial edema may be much more important. Infections and other stressful conditions may precipitate stroke, since increased metabolic rate during such episodes may accelerate sickling. A significant reduction of stroke with hydroxyurea may also suggest that a significant proportion of strokes are secondary to the increased WBC and PLT counts induced disseminated capillary inflammation and edema (16,48).

As a conclusion, SCDs are chronic destructive processes on endothelium particularly at the capillary level, and terminate with accelerated atherosclerosis induced end-organ failure in early years of life. Probably digital clubbing is one of the terminal consequences of the SCDs indicating significantly shortened survival in such patients.

References

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365: 1415-1428.
2. Helvaci MR, Kaya H, Duru M, Yalcin A. What is the relationship between white coat hypertension and dyslipidemia? Int Heart J 2008; 49: 87-93.
3. Helvaci MR, Kaya H, Seyhanli M, Yalcin A. White coat hypertension in definition of metabolic syndrome. Int Heart J 2008; 49: 449-457.
4. Helvaci MR, Sevinc A, Camci C, Yalcin A. Treatment of white coat hypertension with metformin. Int Heart J 2008; 49: 671-679.
5. Helvaci MR, Kaya H, Yalcin A, Kuvandik G. Prevalence of white coat hypertension in underweight and overweight subjects. Int Heart J 2007; 48: 605-613.
6. Helvaci MR, Aydogan A, Akkucuk S, Oruc C, Ugur M. Sickle cell diseases and ileus. Int J Clin Exp Med 2014; 7: 2871-2876.
7. Helvaci MR, Acipayam C, Aydogan A, Akkucuk S, Oruc C, Gokce C. Acute chest syndrome in severity of sickle cell diseases. Int J Clin Exp Med 2014; 7: 5790-5795.

8. Mankad VN, Williams JP, Harpen MD, Manci E, Longenecker G, Moore RB, et al. Magnetic resonance imaging of bone marrow in sickle cell disease: clinical, hematologic, and pathologic correlations. Blood 1990; 75: 274-283.
9. Castro O, Brambilla DJ, Thorington B, Reindorf CA, Scott RB, Gillette P, et al. The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. Blood 1994; 84: 643-649.
10. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease 2010. Global initiative for chronic obstructive lung disease (GOLD).
11. Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, et al. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. Am J Respir Crit Care Med 2009; 179: 615-621.
12. Vandemergel X, Renneboog B. Prevalence, aetiologies and significance of clubbing in a department of general internal medicine. Eur J Intern Med 2008; 19: 325-329.
13. Schamroth L. Personal experience. S Afr Med J 1976; 50: 297-300.
14. Helvaci MR, Aydin LY, Aydin Y. Digital clubbing may be an indicator of systemic atherosclerosis even at microvascular level. HealthMED 2012; 6: 3977-3981.
15. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. JAMA 2014; 312: 1033-1048.
16. Helvaci MR, Aydin Y, Ayyildiz O. Hydroxyurea may prolong survival of sickle cell patients by decreasing frequency of painful crises. HealthMED 2013; 7: 2327-2332.
17. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994; 330: 1639-1644.
18. Mathers CD, Sadana R, Salomon JA, Murray CJ, Lopez AD. Healthy life expectancy in 191 countries, 1999. Lancet 2001; 357: 1685-1691.
19. Helvaci MR, Ayyildiz O, Gundogdu M. Gender differences in severity of sickle cell diseases in non-smokers. Pak J Med Sci 2013; 29: 1050-1054.
20. Myers KA, Farquhar DR. The rational clinical examination. Does this patient have clubbing? JAMA 2001; 286: 341-347.
21. Fodor JG, Tzerovska R, Dorner T, Rieder A. Do we diagnose and treat coronary heart disease differently in men and women? Wien Med Wochenschr 2004; 154: 423-425.
22. Helvaci MR, Aydin Y, Gundogdu M. Smoking induced atherosclerosis in cancers. HealthMED 2012; 6: 3744-3749.
23. Grunberg NE, Greenwood MR, Collins F, Epstein LH, Hatsukami D, Niaura R, et al. National working conference on smoking and body weight. Task Force 1: Mechanisms relevant to the relations between cigarette smoking and body weight. Health Psychol 1992; 11: 4-9.
24. Walker JF, Collins LC, Rowell PP, Goldsmith LJ, Moffatt RJ, Stamford BA. The effect of smoking on energy expenditure and plasma catecholamine and nicotine levels during light physical activity. Nicotine Tob Res 1999; 1: 365-370.
25. Hughes JR, Hatsukami DK. Effects of three doses of transdermal nicotine on post-cessation eating, hunger and weight. J Subst Abuse 1997; 9: 151-159.
26. Miyata G, Meguid MM, Varma M, Fetissov SO, Kim HJ. Nicotine alters the usual reciprocity between meal size and meal number in female rat. Physiol Behav 2001; 74: 169-176.
27. Laaksonen M, Rahkonen O, Prattala R. Smoking status and relative weight by educational level in Finland, 1978-1995. Prev Med 1998; 27: 431-437.
28. Helvaci MR, Kaya H, Gundogdu M. Association of increased triglyceride levels in metabolic syndrome with coronary artery disease. Pak J Med Sci 2010; 26: 667-672.
29. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998; 279: 1477-1482.
30. Mannino DM, Watt G, Hole D, Gillis C, Hart C, McConnachie A, et al. The natural history of chronic obstructive pulmonary disease. Eur Respir J 2006; 27: 627-643.
31. Mapel DW, Hurley JS, Frost FJ, Petersen HV, Picchi MA, Coultas DB. Health care utilization in chronic obstructive pulmonary disease. A case-control study in a health maintenance organization. Arch Intern Med 2000; 160: 2653-2658.
32. Anthonisen NR, Connett JE, Enright PL, Manfreda J; Lung Health Study Research Group. Hospitalizations and mortality in the Lung Health Study. Am J Respir Crit Care Med 2002; 166: 333-339.
33. McGarvey LP, John M, Anderson JA, Zvarich M, Wise RA; TORCH Clinical Endpoint Committee. Ascertainment of cause-specific mortality in COPD: operations of the TORCH Clinical Endpoint Committee. Thorax 2007; 62: 411-415.
34. Helvaci MR, Erden ES, Aydin LY. Atherosclerotic background of chronic obstructive pulmonary disease in sickle cell patients. HealthMED 2013; 7: 484-488.
35. Minniti CP, Eckman J, Sebastiani P, Steinberg MH, Ballas SK. Leg ulcers in sickle cell disease. Am J Hematol 2010; 85: 831-833.
36. Trent JT, Kirsner RS. Leg ulcers in sickle cell disease. Adv Skin Wound Care 2004: 17; 410-416.
37. Helvaci MR, Sevinc A, Camci C, Keskin A. Smoking and sickle cell diseases. Exp Clin Cardiol 2014; 20: 3706-3722.
38. Xia M, Guerra N, Sukhova GK, Yang K, Miller CK, Shi GP, et al. Immune activation resulting from NKG2D/ligand interaction promotes atherosclerosis. Circulation 2011; 124: 2933-2943.
39. Mostafa A, Mohamed MK, Saeed M, Hasan A, Fontanet A, Godsland I, et al. Hepatitis C infection and clearance: impact on atherosclerosis and cardiometabolic risk factors. Gut 2010; 59: 1135-1140.
40. Bonora E, Targher G. Increased risk of cardiovascular disease and chronic kidney disease in NAFLD. Nat Rev Gastroenterol Hepatol 2012; 9: 372-381.

41. Anderson RN, Smith BL. Deaths: leading causes for 2001. Natl Vital Stat Rep 2003; 52: 1-85.
42. Helvaci MR, Sevinc A, Camci C, Keskin A. Atherosclerotic background of cirrhosis in sickle cell patients. Pren Med Argent 2014; 100: 127-133.
43. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. N Engl J Med 2014; 371: 699-710.
44. Gueguen A, Mahevas M, Nzouakou R, Hosseini H, Habibi A, Bachir D, et al. Sickle-cell disease stroke throughout life: a retrospective study in an adult referral center. Am J Hematol 2014; 89: 267-272.
45. Majumdar S, Miller M, Khan M, Gordon C, Forsythe A, Smith MG, et al. Outcome of overt stroke in sickle cell anaemia, a single institution's experience. Br J Haematol 2014; 165: 707-713.
46. Helvaci MR, Aydogan F, Sevinc A, Camci C, Dilek I. Platelet and white blood cell counts in severity of sickle cell diseases. Pren Med Argent 2014; 100: 49-56.
47. Kossorotoff M, Grevent D, de Montalembert M. Cerebral vasculopathy in pediatric sickle-cell anemia. Arch Pediatr 2014; 21: 404-414.
48. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med 1995; 332: 1317-1322.