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Results of Transcranial Doppler in Children with Sickle Cell Disease: Correlation between the Time-Averaged Mean of Maximum Velocity and Some Hematological Characteristics

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Abstract

Background: Transcranial Doppler ultrasonography results have not been previously studied in among Iranian sickle cell anemia patients. The present study was performed to evaluate the pattern of intracranial flow velocities among Iranian children with sickle cell anemia and the hematological parameters that can affect the time-averaged mean of maximum velocity in major intracranial arteries.

Materials and methods: A total of 34 patients with sickle cell anemia aged 2-16 years (Mean: 9.5 ±4.1 years) were enrolled in this cross sectional study. A special pediatric Doppler transducer was used to study intracranial vessels such as left and right middle cerebral arteries and the time-averaged mean maximum velocity was determined for major intracranial arteries on each side. The results of transcranial Doppler were studied according to the Stroke Prevention Trial in Sickle Cell Anemia criteria.

Results: In total a relatively high transcranial Doppler value was found in 9 patients (26.4%) on either right or left side. Out of these 5 patients (14.7%) were in low risk zone (140-169 cm/sec), 3 patients (8.8%) in conditional zone (170 to 200 cm/sec), and one patient (2.9%) in high risk zone (>200cm/sec). Time averaged mean of maximum velocity on the right side and the left side had inversely significant correlation with Hb F (p=0.03) and Hb S (p=0.02).

Conclusion: Transcranial Doppler parameters in patients with sickle cell anemia in our study indicated about one forth of patients to have degrees of increased intracranial flow velocity. In contrast to most other studies the cerebral blood flow velocities among our patients were not significantly correlated with most haematological parameters except for Hb F and Hb S.

Key Words: Sickle cell anemia, transcranial, Doppler, children, Iran.

Introduction

Stroke is a serious complication of sickle cell disease (SCD). The frequency of stroke in SCD is es¬timated as 0.61 per 100 patients per year . The majority of strokes are ischemic events caused by stenosis of large cerebral arteries [1]. Stroke generally occurs without warning and causes significant long-term neurologic sequelae in at least 50% of cases. Regular transfusion after a first stroke strikingly reduces the occurrence of re-stroke [2].

Transcranial Doppler (TCD) is a valuable screening test in many clinical conditions such as SCD, to identify the patients at risk of stroke. TCD uses ultrasound waves to penetrate into skull and assess the speed of blood flow. An abnormally high blood speed is an indicator of a narrowed cerebral blood vessel and/or increased blood volume and a warning marker of high risk for stroke. TCD is easy to perform, painless, non-invasive, inexpensive and free of side effects, so it is a useful measurement tool in children.

Regular blood transfusion and treatment with hydroxyurea can significantly reduce the incidence of stroke in children with SCD who are found to be at high risk of stroke based on abnormal TCD

findings [3, 4, 5]. Previous findings has demonstrated that routine TCD screening test in children with SCD can indicate the high risk group and is useful in recommendig the initiation of regular blood transfusion or alternatively use of hydroxyurea [5].

The objective of the present study was to evaluate the pattern of intracranial flow velocities among Iranian children with SCD and also the hematological parameters that can affect the time averaged mean of maximum velocity (TAMMV) in major intracranial arteries.

Materials and methods

This study was approved by the Research Review Board of Jundishapur University of Medical Sciences and Ahvaz Research Center for Thalassemia and Hemoglobinopathy. Informed consent was obtained from all patients and/or their parents. A comprehensive questionnaire which included questions about the demographic characteristics, signs and symptoms, history of transfusion, hydroxyurea taking and laboratory parameters were designed and filled in for every patient. Thirty four patients with SCD aged 2-16 years and 34 normal volunteers, with the same ethnicity and matched age and sex as the control group, were recruited in the present cross sectional study. The study group was drawn from the Research Center for Thalassemia and Hemoglobinopathy at Shafa Children's' Hospital, Ahvaz, Iran, by following inclusion criteria: age 2 to 16 years; definite diagnosis of SCD in steady state and no history of stroke. Exclusion criteria were: history of major head injury, history of seizure disorder, history of blood transfusion or hydroxyurea therapy, occurrence of acute chest syndrome or acute illness and pain crisis in the period of sonographic testing, history of prenatal or perinatal hypoxic-ischemic brain injury.

The cerebral blood velocities were measured using a 2-MHZ, power motion colour Doppler (Multi-Dop x4 system, DWL, Germany). The examination was performed in quiet and wakeful patients by the same examiner. Restless and uncooperative children were excluded from the study. The test was performed in supine position and a transtemporal window was used as acoustic window for the TCD probe transducer. A special paediatric transducer was used for measurement of the blood flow velocities in left and right middle cerebral arteries. We measured the blood flow velocities in most great cerebral vessels, but in the present study the left and right middle cerebral arteries are reported since the stenosis and occlusion of middle cerebral arteries (MCA) are the most frequent cause of brain infarction among these patients. Time-averaged mean maximum velocity (TAMMV) was used to determine the TCD values for both right and left middle cerebral arteries. The MCAs were usually obtained at a depth of 40-55 mm. The blood flow changes were documented on two consecutive TCD studies. Ultrasound signal was in accordance with the STOP criteria [6].

Four millilitre blood samples were drawn and collected in ethylene-diamine-tetra-acetic acid (EDTA) for cell blood count and Hb electrophoresis. Hematological parameters were determined using an automated hematology analyser (SYSMEX K-1000, SYSMEX CORPORATION, JAPAN). Hemoglobin electrophoresis was performed and HbF was measured using a high-performance liquid chromatography (D10, Bio-Rad, France, Elitech Kit, France) and alkali denaturation. LDH was measured using an auto-analyzer (BT 3000, Biotemcnica, Italy). Reticulocyte count was performed by supravital staining and microscopic eye observation counting. Solubility test was used to distinguish HbS and HbD in some suspicious cases. DNA was prepared from peripheral blood leukocytes and haplotype analysis was performed using a polymerase chain reaction based approach, studying the following restriction enzyme sites of the β-globin gene cluster: [(1) HincII 5′ to ε (2)XmnI-5′γG, (3) Hind III-γG, (4) Hind III-γA, (5) HincII- ′β, (6) HincII- 3′to Ψβ, and (7) HinfI and RsaI - 5′to β] using the method described by the manufacturer protocol. After amplification and digestion with the respective restriction enzymes, the samples were run on an agarose gel electrophoresis and stained with ethidium bromide for visualizing in an ultraviolet transilluminator.

After collecting data, statistical analysis was performed using SPSS software (Version 16.0, SPSS Co, Chicago IL). P values less than 0.05 were considered statistically significant.

Results

A total of 34 patients with SCD (16 male, 18 female) and 34 normal volunteers as the control group were tested. Laboratory results of patients were as follow: RBC count:3.3±0.8106/µl, WBC

count:11900±6000 × 109/L, MCH:27.5±4.5pg, MCV:84.8±10.6 FL, MCHC:33.1±4.7g/dl, platelets count:377500±189000(×109/l), Hb :8.9 ±1.6g/dl, Hb S:78.8±9.5, Hb F:18.3±9.9%, reticulocyte count:4.3±2.8%, LDH:1338.1±506.9 (IU/ml). All hematologic parameters in control subjects were in normal range.

The analysis of TCD results among the patients according to the STOP protocol were as follow: right time averaged mean of maximum velocity (RTAMMV): 125.8 ±24.4cm/sec (Control: 92.8±11.9) (p=0.001), and left time averaged mean of maximum velocity (LTAMMV): 107.5±28.2 cm/sec (Control: 96.7±10.9) (p=0.04). Most patients had a LTAMMV of lower than 140 cm/sec (91.3%), and the rest of patients were equally divided into three categories of low risk zone (140-169 cm/sec) one patients (2.9%), conditional zone (170 to 200 cm/sec) one patient (2.9%), and high risk zone (>200cm/sec) one patient. RTAMMV also revealed that most patients (82.3%) had a reading lower than 140 cm/sec, four patients (11.8%) were in 140 to169 zone, two patients (5.9%) were in conditional zone (140-169 cm/sec), and there were no patients in the high risk zone. In control group all cases had a reading of less than 140 cm/sec.

The Hb and hematocrite levels were significantly lower in SCD patients in comparison to normal group. The Arab/Indian was the most frequent haplotype, present in 40 percent of chromosomes, followed by Benin haplotype (17%), Senegal haplotype (16%), Bantu haplotype (14%) and Cameroon haplotype (13%).

LTAMMV but not RTAMMV had inversely significant correlation with Hb F (p= 0.02) and Hb S (p= 0.03). We did not found any significant correlation between TCD results and other hematologic parameters such as WBC count, Platelet count, MCHC, Reticulocyte count, LDH and beta chain haplotype.

Discussion

Doppler principles were first used in neurology by Aaslid in 1982 and since then, it has been an attractive method to evaluate the cerebrovascular circulation in patients with neurovascular disorders and SCD as well [7]. Adams and his colleagues have found that patients with SCD have higher baseline TCD flow velocities than age-matched children without sickle cell anemia. These increased TCD velocities were related in part to the anemia and in part to pathological arterial stenosis [3]. Patients with MCA velocity of 200cm/sec or higher on TCD have a 40% stroke risk over 3 years, so TCD might be used as a tool for primary stroke prevention in patients with SCD [3].

A total of 190 children with SCD were screened using TCD by Adams and his colleagues. They followed their patients for an average of 29 months. Twenty three patients had a TAMMV >170 cm/sec, six stroke episodes occurred among these 23 patients with abnormal TCD, but only one stroke attack occurred in patients with normal TCD. Adams et al. confirmed these results in another study. They found a 10% risk for stroke per year in patients with abnormal TCD velocities , compared to a 2% risk in patients with normal TCD [5]. The importance of high initial TCD is the conversion of conditional TCD to the high risk group; which occurs especially in younger patients [8]. Age less than 4 years has been indicated as a significant risk factor for conversion [5].

The present study showed that there was a significant difference in cerebral blood flow velocities between SCD patients and the control group. This result is in accordance with Newell et al. and Melo et al. findings [9, 10].

In a cohort study from the Medical College of Georgia, 36.8% of SCD patients had TCD velocities of 140 cm/sec or higher, 17.5% had velocities of 170 cm/sec or higher and 7.9% had velocities of 200 cm/sec or higher [3, 5], which is somehow higher than the findings in our study.

There was no correlation between TCD velocities values and beta gene haplotypes in our study. Powars et al. showed a significant correlation between beta gene benign haplotypes, Senegal and Arab Indian, with silent infarction. They also reported lower rates of overt stroke with these beta gene haplotypes [11]. Kinney et al. found a positive association with the Senegal haplotype [12].

Some studies have indicated a significant correlation between conditional and abnormal TCD values with age, Hb, leukocyte and reticulocyte counts which is in disagreement with our findings [10, 13, 14]. But Deane et al. did not found any correlation between MCA velocity and Hb level, neutrophil count, platelet count, LDH level, percentage of hemoglobin F and other parameters thought to be important in SCD [15], which is similar

to our findings except for Hb F and Hb S levels which showed an inverse correlation with LTAMMV in our study.

Conclusion

Transcranial Doppler parameters in patients with sickle cell anemia in our study indicated about one forth of patients to have degrees of increased intracranial flow velocity. In contrast to most other studies the cerebral blood flow velocities among our patients were not significantly correlated with most haematological parameters except for Hb F and Hb S.

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