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Detection of Haptoglobin 1, 2 Gene Polymorphisms in Sickle Cell Disease Saudi Children Patients.

KEYWORDS: SCD, Haptoglobin , Gene polymorphism, Saudi children patients

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ABSTRACT *Background: Sickle cell disease (SCD) is an inherited blood disorder. It is most often found in people with African heritage, but it can also be found in people with ancestry from other parts of the world. To understand this condition, it helps to know more about how your blood is made.*

Objectives: This study aimed to determine the prevalence of haptoglobin (Hp) 1, 2 gene alleles in Saudi sickle cell disease (SCD) patients.

Subjects and methods: Hundred SCD Saudi children patients were included in this study and 50 normal individuals were selected as control group. 5 ml of venous blood was collected from every SCD patient into EDTA test tubes and used to determine the Hp genotyping using a PCR technique followed by agarose gel electrophoresis.

Results: The frequency of the Hp-1 allele was 30.8% among SCD Saudi children patients, while the Hp-2 allele predominated with (60.7%). However, the differences were not significant (p 1 0.05) when the allele distributions were compared between SCD Saudi children patients and AA control participants.

Conclusions: These findings suggest that the frequency of Hp1 and Hp 2 appears to follow ethnic and geographical distribution.

Introduction

Sickle cell disease is an inherited blood disorder. It is most often found in people with African heritage, but it can also be found in people with ancestry from other parts of the world. To understand this condition, it helps to know more about how your blood is made[1].

Your blood contains millions of red blood cells. Each of your red blood cells has hemoglobin, which gives blood its red color and carries oxygen throughout your body2. Hemoglobin is made by combining a "heme" portion (iron) and a "globin" portion (protein). The iron comes from the food you eat and your body makes the globin[3].

There are different kinds of hemoglobin that the body can make. The most common kind in an adult is hemoglobin A. For hemoglobin A, your body puts two "alpha" globin chains together with two "beta" globin chains. Sickle cell happens when there is a difference in how the beta globin chains are made[2].

The instructions for making globin chains are part of the genetic information you inherit from your parents. Genetic instructions are called genes. You inherit your genes in pairs, with one copy of each gene coming from each parent. One particular gene, the beta globin gene, is responsible for telling the body how to make beta globin chains. Sickle cell disease happens when both copies of the beta globin genes are not working in the usual way.

In sickle cell disease, the body makes hemoglobin S instead of the most common kind of hemoglobin, hemoglobin A. Although hemoglobin S is able to carry oxygen around in the blood, a slight chemical difference makes it more likely to collapse into a sickle (banana-like) shape instead of the usual round (donut-like) shape. This makes the red blood cells more rigid and sticky. A person with one normal copy of the beta globin gene and one copy making hemoglobin S has sickle cell trait. A person with sickle cell trait makes a small amount of hemoglobin S, but also makes plenty of hemoglobin A. About 10% of the African-American population has sickle cell trait[5].

A person with sickle cell trait will never develop sickle cell disease and usually has no medical problems from the trait. Very rarely, if you have sickle cell trait, your blood cells can sickle (change shape) when your body is not getting enough oxygen, such as during travel to high altitudes or when doing very strenuous exercise.

The importance of identifying sickle cell trait is that it helps find couples whose children may be born with sickle cell disease[6].

Sickle cell disease is a lifelong condition that can include serious health problems, but it affects each person differently. When the blood cells become sickle-shaped, they can get stuck in the blood vessels and create blockages. This leads to pain in the area of the blockage and may cause damage to that area. Bones are very often affected, but it can happen in any part of the body, like the spleen, liver, heart, lungs, kidney, brain, and muscles. Sickled cells also get broken

down more quickly by the body, which causes chronic anemia and fatigue.

Most of the time sickle cell disease happens when both of the beta globin genes are making hemoglobin S, instead of hemoglobin A. This is called hemoglobin SS disease or sickle cell anemia. But there are changes in the beta globin gene that lead to other differences in hemoglobin, such as hemoglobin C, hemoglobin D, hemoglobin E, and beta thalassemia. When one of these other hemoglobin traits combines with hemoglobin S, the result is a form of sickle cell disease. The less common types of sickle cell disease are hemoglobin SC disease, hemoglobin SD or SE disease, and hemoglobin S-beta thalassemia disease.

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Some types of sickle cell disease have more medical problems than others[7].

Sickle cell disease can only happen when both parents have sickle cell trait (or a related blood trait). When both parents have sickle cell trait, there is a 25% (or 1 in 4) chance in each pregnancy for the baby to have sickle cell disease and a 75% (or 3 in 4) chance that the baby will not have this disease.

The first-line scavenger of free plasma Hb is haptoglobin (Hp), to which it is rapidly bound following its release. The Hb-Hp complex exposes a neoepitope that is recognized-by the Hb scavenger receptor, CD163, on the surface of monocytes and macrophages through which thecomplexes are endocytosed and degraded 10. Hp is an_ -sialoglycoprotein found in all mammals, but exhibiting a polymorphism only in humans, in whom 3 major functional phenotypes have been described: Hp 1-1,Hp 2-2 and the heterozygous Hp 2-1. Hp 1-1 is the most biologically active in binding free Hb and suppressing consequent inflammatory responses; Hp 2-2 is the least There is marked variation in the frequency of HP genes with geographic region7. The HP2 allele originated in India ~2 mya and propagated around the world as a result of intense genetic pressure, gradually replacing the hegemony of the HP1 allele. This suggests that the HP2 allele may have a selective advantage over the HP1 allele7. The frequency of the HP1 allele increases from Southeast Asia to Europe and Africa, and from Asia to America, by way of Alaska. In America, the highest frequencies are found in indigenous populations of Chile, Peru, Mexico, Venezuela and on the Brazilian-Venezuelan border, among the Yanomama Indians.[7]

The equilibrium of the HP1/HP2 polymorphism is broadly constant throughout the world. The allele frequencies in European populations are ~0.43 for the HP1 allele and 0.57 for the HP2 allele; in American populations, the corresponding figures are ~0.54 and 0.46 (Langlois and Delanghe, 1996). Recent studies of populations from southern and southeastern Brazil have revealed allele frequencies of ~0.53 and 0.46 for HP1 and 0.47 and 0.54 for HP2, respectively (Souza et al., 2003; Zaccariotto et al., 2006). Shreffler and Steinberg (1967) found frequencies of 0.48 and 0.47 for HP1 and 0.52 and 0.53 for HP2 among Xavante Indians living in the villages of Simões Lopes and São Marcos, respectively, in central-western Brazil. More recently, Simões et al. (1989) found very high frequencies of the HP1S allele and low frequencies or complete absence of the HP1F allele among Macushi and Içana River Indians in the Amazon region. Table 1 summarizes several studies that have examined the frequency of HP alleles in different populations around the world[8].

Materials and Methods.

Hundred SCD Saudi children patients and fifty AA controls were enrolled in this study. Routine complete blood cell counts and electrophoresis were carried out . DNA-was extracted with the method of Poncz *et al.* [23] and the _ S haplotype determined using standard techniques [24] . DNA samples were stored frozen at –70 ° C . All SS samples were screened for _S haplotypes; Hb AA siblings were used as controls.

Hp Genotyping.

Hp genotypes were characterized using PCR amplification of DNA segments representing the Hp-1 and Hp-2 alleles, followed by agarose gel electrophoresis [25]

The frequencies of the genotypes and individual alleles are presented as percentages. Comparisons were made within and betweengroups using the X^2 test

Results.

Table (1) Electrophoresis result for SCD Saudi children patients and control.

Type of sample	Number of sample	Type of Hb	%
SCD patients	100	SS	100
Control	50	AA	100

Table (2) Frequency of Hp allels in SCD Saudi children patients

Type of Hb	Hp 1	Hp 2	Total
SCD (SS)	26	74	100
Normal control (AA)	32	28	50

Table 3.Frequencies of Hp polymorphisms

Genotype	SCD Saudi children patients	Normal AA
2-2	55	26
2-2	42	23
1-1	3	2
Total	100	50

Discussion

In clinical settings, the haptoglobulin assay is used toscreen for and monitor intravascular hemolytic anemia. In intravascular hemolysis, free hemoglobin will be released into circulation and hence haptoglobin will bind the hemoglobin. This causes a decline in haptoglobin levels. Conversely, in extravascular hemolysis the reticuloendothelial system, especially splenic monocytes, phagocytose the erythrocytes and hemoglobin is not released into circulation; serum haptoglobin levels are therefore normal. Frequencies of the Hp1 and Hp2 phenotypes have been reported to exhibit geographic differences that are dependent on ethnicity [(2)]. Blackwell and The phusdin conducted a 'pheonotypic' study involving 682 healthy adults and reported that the frequency of theHp1 phenotype among Thais was ;0.24%, and 2.3% of Thai individuals have the Hp 0-0 phenotype [(13).]Expression of the Hp gene is absent in an haptoglobinemia (Hp 0-0 phenotype); a condition present in 1 in 1000 Caucasians. In blacks, especially of West African descent (i.e., Nigeria and Cameroon),

Teye et al. reported that the A–61C mutation in the promoter of the Hp gene and another mutation in exon 7 in the b-chain of the Hp2 allele reduced transcription activity and Hp gene expression [(17, 18)]. In addition, these mutations seem to be related to some of the anhaptoglobinemic individuals in Africa (19). No other detectable change in the Hp gene clusters, including the promoter region, of Hp0 individuals has been reported in other areas. Some authors presume that the reduced expression of Hp is related to the trans-acting factors necessary for Hp expression, or cis-acting promoter sequences [(20)]. However, at present there is no apparent research to confirm the speculation in other areas. Blackwell et al. [(8)] Also 748 Su et al.: Hpdel in Taiwan Article in press - uncorrected proof investigated the Hp phenotype among Taiwanese. Unfortunately, their method was not a genetic survey by PCR but rather a genotypic survey by electrophoresis. Thus, the individuals with the heterozygous allele of Hp0 (Hp1-0 and Hp 2-0) could be misidentified as Hp 1-1 or Hp 2-2. In our series, the Hp1 allele frequency (33%) was nearly the same as previously reported by Blackwell (28%). However, the Hp0 frequency as determined by the PCR method was more accurate in detecting individuals with Hp0 heterozygosity. Neverthe-

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less, differentiating between congenital and acquired hypohaptoglobinemia could be difficult. There are many factors that may interfere with the diagnosis of hypohaptoglobinemia, including age, underlying congenital diseases such as hemolytic disorders (e.g., hereditary red cell membrane and enzyme defects, thalassemia, and sickle cell anemia), and even lifestyle factors (e.g., repetitive physical exercise associated with limited mechanical trauma to erythrocytes). No Hp can be detected in neonatal serum. However, by the sixth month of life, failure to detect Hp becomes relatively rare. About 75% of Hp0 subjects with Plasmodium vivax infection, when treated with chloroquine, showed typable Hp polymorphs by 8–9 days post-treatment [21].

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