Niger. J. Physiol. Sci. 33(June 2018)025-029
www.njps.com.ng

Niger. J. Physiol. Sci.

Ahaptoglobinaemia in a Nigerian Cohort

[1]Chidiebere Chidubem Eluke, [2]*Blessing Chekwube Eluke, [3]Ndidiamaka Ikechukwu Okereke, [2]Chukwugozie Okwuosa, [2]Silas Ufelle

[1]Department of Morbid Anatomy, Faculty of Medicine and Dentistry, College of Medicine, University of Nigeria, Enugu campus, Nigeria, [2]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Medicine University of Nigeria, Enugu Campus. Enugu Nigeria, [3]Department of Haematology, Federal Teaching Hospital, Abakiliki Ebonyi State

Summary: Ahaptoglobinaemia have been indicated in blacks from West Africa. Owing to the clinical and biologic importance of haptoglobin (hpt), this work explores the situation in a Nigerian cohort since there are no published values of haptoglobin levels of individuals in this locality. The study was aimed at determining the amount of haptoglobin in the blood of normal healthy Nigerians. Haptoglobin was quantitatively estimated in one hundred and fifty-two apparently healthy individuals using highly sensitive immunoassay technology. Blood grouping and haemoglobin genotype were assayed for all subjects to know if they influence haptoglobin levels. The association between haptoglobin and blood group was also established. Serum levels of haptoglobin among all subjects analyzed revealed a marked decrease in their haptoglobin levels when compared to other reference intervals. A further association between haptoglobin and gender did not reveal a statistical significant relationship ($p>0.05$). However, there was a significant different when haptoglobin levels of different blood groups and haemoglobin genotype when compared. Our data suggest that serum levels of haptoglobin are significantly lower in healthy Nigerians. The lower limit was remarkably lower than the internationally acceptable Caucasian reference range suggesting a clear necessity for establishing reference African values.

Keywords*:* Haptoglobin, Nigeria, Ahaptoglobinaemia, reference interval

**Address for correspondence*: blessingeluke@gmail.com PHONE: 08032641174

Manuscript Accepted: February, 2018

INTRODUCTION

Haptoglobin (Hpt) is an α2 glycoprotein that irreversibly binds free haemoglobin released from lysed red cells. The free haemoglobin forms a haptoglobin – haemoglobin complex which is removed by the reticulo-endothelial system (Lim *et al.*, 2008). This protein is characterized by a genetic polymorphism of two autosomal co-dominant genes Hpt1 and Hpt2 which results in three phenotypic variants; Hp1-1, Hp 2-1 and Hp 2-2 (Smithes, 1955, Smithes *et al.*, 1955). Hpt is also an acute phase protein synthesized primarily by the liver cells as a response to the production of IL6 and TNF. Apart from its function as a laboratory marker for the diagnosis of haemolytic anaemia, it serves other biologic functions which include; modulation of immune response, formation of new blood vessels in conditions such as wound healing, systemic vasculitis and tumor growth (Langlois et al 1996). It has antioxidant ability and protects against toxic radicals. Free iron and haemoglobin can cause the oxidation of low density lipoprotein by the generation of hydroxyl or superoxide radicals (Grinshtin *et al.*, 2003). Haptoglobin can reduce the damage caused by free radicals to vascular endothelial cells. Furthermore, free haemoglobin released from intravascular haemolysis can cause damage to the renal tubules if haemoglobin is not cleared by hepatocytes after forming a complex with haptoglobin (Sandrzadeh *et al.*, 1999). Haptoglobin is also involved in angiogenesis, bioavailability of nitric oxide and endothelial relaxation and may be involved in the suppression of lymphocyte function (Langlois *et al.*, 1996).

However, functional and structural differences that exist between the different phenotypes may have marked implications in biologic, clinical variations and disease outcomes (Braeckman et al 1999, Woben et al 2008). Evidence has linked frequency of these genes to geographic location (Carter et al 2007, Kasvosve et al 2000). Furthermore, recent studies have associated haptoglobin gene polymorphism with the initiation and development of some disease processes and pathways (Fowkes et al., 2006; Atkinson et al., 2007). Disorders such as atherosclerosis, chronic inflammatory disease and infections have been implicated (Mohieldein et al., 2012; Quaye, 2008). Recent studies have analyzed the effect of haptoglobin polymorphism on serum cholesterol and albumin concentration, high density lipoprotein triglycerides and serum ceruloplasmin. Although the precise reason

is arguable, Hpt 2-2 was found to have more angiogenic property than other phenotypes (De Kleijn, 2002). Furthermore, other works have linked Hpt1 with more protective ability against oxidation and peroxidation (Dobryszycka, 1997). Since increased levels of free haemoglobin will lead to markedly reduced haptoglobin serum levels, finding the reference range of a particular population becomes vital. More especially when studies are trying to find reasons for their increase in some diseases. Some studies have demonstrated that haptoglobin influenced conditions like cancer, some infectious diseases, epileptic seizures, atherosclerosis and diabetes (Delanghe, 1995). Other reports have emphasized the relationship between haptoglobin phenotypes and increased susceptibility to disease development (Levy et al., 2002).

Increasingly, haptoglobin polymorphism is linked to modifications in disease presentation and associated with other systems example blood group and haemoglobin types (Dobryszycka, 1997). Furthermore, a recognized phenotype Hp O characterized by absence or reduced production of haptoglobin gene results primarily in decreased levels of serum concentration called ahaptoglobinaemia (Kirk et al., 1970; Constans, 1981). It is believed that blacks have different levels of haptoglobin when compared to Caucasians (Teye et al., 2003; Mastana et al., 1994). Some studies compared the frequency of haptoglobin gene distribution in various countries (Shim et al., 1964; Allison et al., 1958). More than 30% of blacks in West Africa were reported with increased frequency of ahaptoglobinaemia (Park et al., 2004; Trape et al., 1988). Owing to the importance of haptoglobin in medicine and the paucity of documented scientific information in the case of Nigeria, this study attempts to determine the distribution of haptoglobin levels in serum of normal individuals in Nigeria.

MATERIALS AND METHODS

One hundred and fifty-two apparently healthy Nigerians in Enugu State were enrolled in the cross sectional study between March and August 2016. The subjects were of both sexes and were aged between 25 to 45years. Following approval by the ethics committee and review board of the University of Nigeria Teaching Hospital Ituku- Ozalla, informed consent was obtained from each person using approved protocol. All subjects and protocol were handled according to guidelines as outlined by Helsinki declaration. Exclusion criteria included those with infections; viral (human immunodeficiency virus, hepatitis B surface antigen, hepatitis C virus), bacterial, parasitic infestation, pregnancy, lactating women and history of illness for two to one month. Blood samples were collected from all inclusive subjects at enrollment for laboratory assays: haptoglobin, blood group and haemoglobin typing according to procedure as described by Dacie and Lewis (Dacie et al., 2006). Blood group and haemoglobin types were determined by serologic analysis and haemoglobin electrophoresis.

Collection of blood

Seven mililitres of blood was drawn from each participant and four mililitres was transferred into EDTA for the assessment of blood group and haemoglobin typing. Four milliliters was dispensed into a sterile plain tube, allowed to clot and serum expressed for the estimation of haptoglobin levels.

Measurement of haptoglobin levels

Serum levels of haptoglobin were determined in duplicates using commercially available sandwich Enzyme Linked Immunosorbent Assay (ELISA)kits by My Biosource San Diego CA. A high sensitive well calibrated ELISA technique was used due to availability. ELISA is a technique that may be used in lieu of immunoturbidometry and is commonly available for assays in this part of the world-resource poor country. A high sensitive and specific assay kit with no known cross reactivity or interference between other analogues and Hpt was used. The assay employed microtiter plates coated with polyclonal antibody as capture antibody and biotin conjugated antibody as detecting antibody. The detection range was between 0.312-4000ng/ml while the minimum detectable dose was <0.108ng/ml. the coefficient of variation for between run studies and within run was less 10%.

Methods for blood grouping and haemoglobin genotype

Whole blood was used for the determination of blood groups and haemoglobin type using antigen and antibody agglutination technique. High reactive anti-sera by Lorne laboratories UK was employed for ABO and Rhesus typing using standard tube technique while haemoglobin electrophoresis was used for haemoglobin typing according to techniques by Dacie and Lewis.

Statistical Analysis

Data was analyzed using statistical package for social science -SPSS {version 20} software. Shapiro- Wilk test was used to test for normality to ascertain population distribution. Frequency histogram is shown for population distribution. Statistical significance was calculated using non parametric technique Mann Whitney for sex comparison, Kruskal-Wallis test for blood group comparism. T -test was employed in the comparison with Caucasian value. Relationship between sex/blood group/genotype and haptoglobin was analyzed using Spearman correlation. The haptoglobin levels were measured in µg/ml and the result established with the values at median (50^{th}

percentile), 2.5th and 97.5th percentile. 2.5th and 97.5th percentile values from the population were recorded as the upper and lower reference limit.

RESULTS

The levels of haptoglobin in normal individuals in Nigeria, a country in West Africa are presented in Table 1. The mean haptoglobin level for 152 apparently healthy individuals was 61.59 ± 17.0 µg/ml. The reference interval for haptoglobin level was determined to be 25.90 - 92.29 µg/ml for 2.5 and 97.5 percentiles; median 61.65 µg/ml. The mean haptoglobin level of males (58%) was 63.21(95% Cl= 26.35-94.36 µg/ml) while that for females (42%) was 59.72 (95% Cl= 25.53-91.12 µg/ml).

Table 1 Serum concentration of haptoglobin (µg/ml) in normal adult subjects

	Haptoglobin (µg/ml)	Minimum	Maximum	Median
Total	61.59±1.38	25.04	95.14	61.65
Males (90)	62.3±17.2	25.9	95	69.2
Females (62)	59.6±15.8	25	87	62.9

Table 2 Serum levels of haptoglobin (µg/ml) according to blood group

BLOOD GROUP	Haptoglobin (µg/ml)
A+	70. 39±4.23
B+	57.29±9.09
O+	58.29±1.85
AB+	82.29±4.75

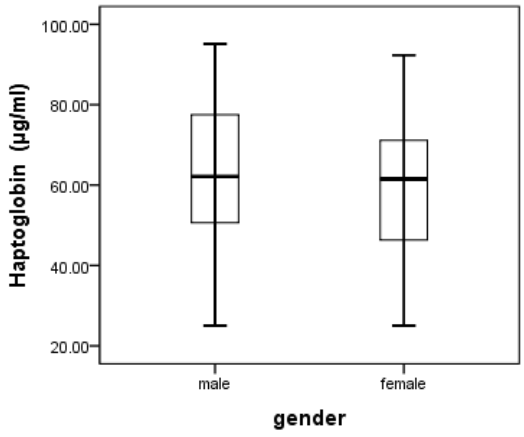

Figure 1 box plot showing relationship between gender and haptoglobin level

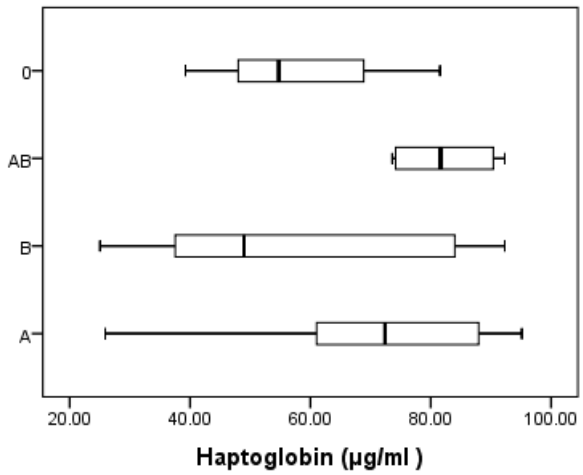

Figure 2 box plot showing relationship between haptoglobin and blood group

The Mann Whitney U test revealed no notable statistical significant difference in haptoglobin levels between sex (p= 0.760). Table 2 shows the haptoglobin levels charted according to blood group of all participants. A Kruskal Wallis technique was used to evaluate the differences between haptoglobin and different blood types(95%Cl). There was a statistically significant difference between blood group and haptoglobin level (X^2 = 12.25, P = 0.007) and between haemoglobin type and haptoglobin (X^2 = 23.60, P = 0.014). Blood group AB (74.6µg/ml) had the highest levels while B blood group had the lowest levels. Hb AA had the highest hpt level and HbSS had the lowest. When the relationship was sought between haptoglobin and sex (r=-0. 081, p>0. 05), there was found to be none.

DISCUSSION

Haptoglobin is an acid phase protein that does not have only an immune-modulatory function but is a potent antioxidant and a known indicator of haemolysis (Langlois et al., 1996). Some studies have attributed low levels of serum haptoglobin to various populations and pointedly to Africa (Trape et al., 1988). These works have implied that the functional differences between various genetic phenotypes lead to varied influence on human pathology. Other studies suggested that particular haptoglobin phenotype are prevalent in blacks (Constans, 1981). Confounding factors like hemolysis, time of collection of blood samples and duplicate analysis were taken into consideration during the design of this work. This study was done to establish the serum levels of haptoglobin from a Nigerian population; a country in tropical West Africa. We found that serum levels of haptoglobin in the study population were lower than that reported for Caucasians (Allison et al., 1958).

Both the lower limit and the upper limit were significantly decreased when compared to known international reference limit which is reported to be 300 - 2150 µg/ml (Kasvosve et al., 2000). The normal range found in this study was between 25 to 92µg/ml. This means that the levels of serum haptoglobin were quite markedly reduced in the study population. Decreased levels of haptoglobin in blood, hypoglobinaemia and ahaptoglobinaemia may be acquired as a result of association with malaria endemicity or may be congenital due to a deletion in the promoter region of haptoglobin gene (Boreham et al., 1979; Shinton et al., 1995). It has been reported by various works that malaria endemicity may directly correlate with the serum levels of haptoglobin and therefore may be linked to ahaptoglobinaemia (Imrie et al., 2006). It is probable that increased clearance of the haptoglobin- haemoglobin complex during malaria parasitaemia may have resulted in the observed levels. Nigeria is a known malaria endemic country. Also, hpt levels have been reportedly influenced by their phenotypes (Atkinson et al., 2007; Hunt et al., 2001). Researches from other works have linked hpt polymorphism and phenotypic variations to the differences in hpt blood levels and invariably to susceptibility to disease outcomes. We intend to see the implication in the Nigerian situation subsequently in further research.

The reduced serum levels of haptoglobin found in this study agrees with some results from other African countries: Congo, Zaire, East Africa, Gambia and South Africa (Allison et al., 1960; Boreham et al., 1979). Apart from genetic factor which have been attributed to ahaptoglobinaemia in some populations, other authors have linked ethnicity and close geographical proximity as the key factor that may influence levels of hpt (Barnicot et al., 1959). Others have argued that it may be purely genetics (Smithes et al., 1955; Woben et al., 2008).

Furthermore, we did not find any significant variations between the haptoglobin level and sex. However, we found significance difference between haptoglobin and blood group. Blood group AB appears to have the highest level of hpt while B blood group has the lowest. The low levels of hpt found in Hb SS may be as a result of haptoglobin – haemoglobin complex formed as a result of free haemoglobin released during haemolysis.

In summary, these results suggest that there was a considerable difference in the reference range of haptoglobin in the study population when compared to the levels established for Caucasians. Already, haptoglobin is associated with the evolution and modification of disease presentation and pathology (Calderon et al., 2006; Nevo et al., 1986). Therefore, knowing the reference interval in a community becomes indispensable and of paramount importance.

REFERENCES

Allison, A.C., Blumberg, B.S., Rees, A.P. (1958). Haptoglobin Types in British, Spanish Basque and Nigerian African Populations. Nature. 181 824 – 825.

Allison AC, Barnicot NA (1960). Haptoglobins and transferrins in some east African peoples. Acta Genet. 1017-1023..

Atkinson., S.H., Mwangi, T.W., Uyoga, S.M., Ogada, E., Macharia, A.W., Marsh, K., et al. (2007). The haptoglobin 2-2 genotype is associated with a reduced incidence of Plasmodium falciparum malaria in children on the coast of Kenya. Clin. Infect. Dis. 44 802-809.

Barnicot, N.A., Garlick, J.P., Singer, R., Weiner, J.S. (1959). Haptoglobin and transferrin variants in Bushmen and some other South African peoples. (Letter). Nature.1842042.

Boreham, P.F., Lenahan, J.K., Boulzaguet, R., Storey, J., Ashkar, T.S., Nambiar, R. et al. (1979). Studies on multiple feeding by *Anopheles gambiae* s.l. in a Sudan savanna area of north Nigeria. Trans. R. Soc. Trop. Med. Hyg. 73 418-423.

Braeckman, L., Bacquer, D.D., Delanghe, J, Claeys, L., Backer, G.D. (1999). Association between haptoglobin polymorphism, lipids, lipoproteins and inflammatory variables. Atherosclerosis. 143 383-388

Carter, K. Worwood, M. (2007). Haptoglobin: A review of the major allele frequencies worldwide and their association with diseases. Int. J. Lab. Hematol. 29 92-110.

Constans, J., Viau, M., Gouaillard, C., Clerc, A., (1981) Haptoglobin polymorphism among Sharian and West African groups. Haptoglobin phenotype determination by radioimmunoelectrophoresis on Hp O samples. Am. J. Hum. Genet. 33 606-616

Dacie, I.V.,, Lewis, S.M., Practical Hematology. 10 th ed. Belfast: University Press; (2006). p. 110.

De Kleijn, D.P., Smeets, M.B., Kemmeren, P.P., Lim, S.K., Van Middelaar, B.J., Velema, E., et al. (2002). Acute-phase protein haptoglobin is a cell migration factor involved in arterial restructuring. FASEB J. 16 1123-1125

Delanghe, J.R., Duprez, D.A., De Buyzere, M.L., Bergez, B.M., Claeys LR, Leroux –Roels et al. (1995). Refractory hypertension is associated with the haptoglobin 2-2 phenotype. J. Cardiovasc. Risk 2 131-136.

Dobryszycka, W. (1997). Biological functions of haptoglobin - New pieces to an old puzzle. Eur. J. Chem. Clin. Biochem. 35;9: 647-654

Fowkes, F.J., Imrie, H., Migot-Nabias, F., Michon, P., Justice, A., Deloron, P, et al. (2006). Association of Haptoglobin levels with age, parasite density, and

haptoglobin genotype in a malaria-endemic area of Gabon. Am. J. Trop. Med. Hyg. 74: 126-130.

Grinshtein, N., Bamm, V.V., Tsemakhovich, V.A., Shaklai N. (2003). Mechanism of low-density lipoprotein oxidation by hemoglobin –derived iron. Biochemistry. 42: 6977-6985.

Kasvosve, I., Gomo, Z.A., Gangaidzo, I.T., Mvundura, E., Saungweme, T., Moyo, V.M. et al. (2000) Reference range of serum haptoglobin is haptoglobin phenotype-dependent in blacks. Clin Chim Acta. 296(1-2) 163-170.

Kirk, R.L., Kinns, H., Morton, N.E. (1970). Interaction between the ABO blood group and haptoglobin system. Am. J. Hum. Genet. 22 384-389

Langlois, M.R., Delanghe, J.R. (1996). Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem. 42 1589–1600.

Levy, A.P., Hochberg, I., JablonskiK, R.H, Lee, E.T., Best L et. al. (2002). Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The strong heart study. JACC. 40: 1984-1990.

Lim, S.K., Ferraro, B., Moore, K. Halliwell, B. (2008). Role of haptoglobin in free hemoglobin metabolism. Redox Rep.6 219–227

Mastana, S.S., Bernal, J.E., Onyemelukwe, G.C., Papiha, S.S. (1994). Haptoglobin subtypes among four different populations. Hum. Hered. 44: 10-13.

Mohieldein, A., Alzohairy, M., Hasan, M., Khan, A.A. (2012). Inflammatory Markers and Haptoglobin Polymorphism in Saudi with Non-insulin-dependent Diabetes Mellitus. Global Journal of Health Science. 5(1) 135-145.

Park, K.U., Song, J., Kim, J.Q. (2004). Haptoglobin genotypic distribution (including Hp0 allele) and associated serum haptoglobin concentrations in Koreans. J. Clin. Pathol. 57: 1094–1095.

Quaye, I.K. (2008). Haptoglobin, inflammation and disease Transactions of the Royal Society of Tropical Medicine and Hygiene. 102: 735—742.

Sandrzadeh, S.M., Bozorgmehr, J. (2004). Haptoglobin phenotypes in health and disorders. Am. J. Clin. Pathol. 121 Suppl S97-104

Shim, B.S, Bearn, A.G. (1964). The distribution of haptoglobin subtypes in various populations, including subtype patterns in some nonhuman primates. Am. J. Hum. Genet. 16: 477-483.

Shinton, N.K., Richarson, R.W., Williams, J.D.F. (1965). Diagnostic value of serum Haptoglobin. J. Clin. Path. 18 114-118.

Smithes, O. (1955). Zone electrophoresis in starch gel : group variations in the serum protein of normal human adult. Biochem. J. 5 : 629-641.

Smithes,O.,Walker, N.F. (1955). Genetic control of some serum proteins in normal humans. Nature. 176 1265-1266.

Teye, K., Quaye, I., Koda Y., Soejima, M., Tsuneoka, M., Pang, H. et al. (2003) A-61C and C-101G Hp gene promoter polymorphism are, respectively, associated with ahaptoglobinaemia and hypohaptoglobinaemia in Ghana. Clin. Genet. 64: 439-443

Trape, J.F., Fribourg-Blancz A. (1988). Ahaptoglobinemia in African populations and its relation to malaria endemicity. Am. J. Epidemio.12 (6) 1282-1288.

Wobeto,V.P., Zaccariotto T.R., Sonati, F. (2008). Polymorphism of human haptoglobin and its clinical importance. Genet. Mol. Biol. 31 (3) 602-620.