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Rarity of Bombay Phenotype Among Blood Donors in Sokoto North Western, Nigeria

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Abstract
Individuals who are Bombay phenotype type as group O on forward ABO typing. Their red cells lack A, B and H antigens and their sera contain anti-A, anti-B, and anti-H. It is important to correctly type individuals who are Bombay phenotype because these individuals require autologous blood donation or blood from another Bombay individual. This study was designed to determine the prevalence of Bombay phenotype among blood donors in Sokoto, North Western Nigeria. Four hundred and fifty three blood group O donors aged 18-55 years (mean age 39 ± 21 years) were typed for the absence of H-antigen using Lorne Diagnostic (UK) anti-H Lectin reagent using the conventional tube technique. Out of the total 453 blood donors tested, 279 were Hausa (61.6%), 42 were Fulani (9.3%), 48 were Hausa/Fulani (10.6%) and 84 (18.5%) were of other ethnic groups. Gender distribution of blood donors indicated that 397 were males (87.6%) and 56 (12.4%) were females. A significant number of blood donors 213(47.0) were in the 26-35 years age group, 111 (24.5%) were in the 36-45 years age group, 108 (23.8%) were in the 16-25 years age group and 21 (4.6%) were in the 26-55 years age group. The prevalence of Bombay phenotype among the blood donor tested was 0.00%. This research demonstrated a 0% prevalence of Bombay phenotype among blood group O donors in Sokoto North Western Nigeria. We recommend that all blood group O blood donors and transfusion recipient be routinely screened for Bombay phenotype to reduce the risk of haemolytic transfusion reaction resulting from a patient with Bombay phenotype being wrongly typed and transfused with blood group O blood. There is need for the implementation of a policy to include serum typing or reverse grouping confirmation along with reaction with 'O' cell control in reverse grouping procedure in blood transfusion laboratories in Nigeria to correlate cell and serum grouping results.

1. Introduction

Blood group determination plays a vital role in transfusion medicine ensuring that recipient receive compatible blood transfusion to prevent haemolytic transfusion reaction (HTR) and Haemolytic Disease of the Foetus and Newborn (HDFN). The Bombay (Oh) phenotype is characterized by the absence of A, B and H antigens on red cells. In the absence of reagents to determine Bombay (Oh) phenotype status of individuals, they can

be potentially be wrongly grouped as group O. Bombay (Oh) individuals have potent anti-H in their plasma which can cause haemolytic transfusion reaction if transfused with H antigen containing group O blood.

Reported prevalence of Bombay phenotype reported in India States of Tamil Nadu and Karnataka was 0.004[1] and 0.005% [2] respectively. The existence of a human H/h genetic polymorphism was first established in Bombay (Mumbai) in India in 1952 [3]. Bombay phenotype individuals are mostly confined to the Southeast Asia [4-5]. A high level of consanguinity has been reported among the parents of individuals with the Bombay phenotype [6]. The Bombay blood group is a rare clinically significant blood group. Individuals of this phenotype often lack the H antigen on the red cell membrane and have potent anti-H in the serum. They fail to express any A, B or H antigen on their red cells or other tissues. Transfusion support for patients with this phenotype should only be from Bombay phenotype donor.

Haemolytic disease of newborn due to Oh phenotype of mother is a rare event and there are very few published reports [7]. Patients with the Bombay phenotype are unique. Their red cells are not agglutinated by anti A and B antisera. Their serum however contains anti A, B as well as strongly reactive anti- H which agglutinates red cells of 'O' group individuals through a wide thermal range [8]. A previous study reported Bombay phenotype in two brothers of North Indian extraction [9]. There is paucity of data on the prevalence of Bombay phenotype in Nigeria. The aim of this study is to determine the prevalence of Bombay phenotype among blood donors of African descent in Sokoto, Nigeria.

1.1. Description of the Study Area

The selected area for this study is Usmanu Danfodiyo University Teaching Hospital (UDUTH) which is located in Wamakko Local Government within Sokoto Metropolitan City. Sokoto is located in North-Western Nigeria. Socio cultural characteristics is homogenous as majority of its indigenes are Muslims, therefore the Muslim religion provides them the code of conduct and behavioral characteristics.

1.2. Study Design

This study was a prospective cross-sectional study aimed at determining the prevalence of Bombay phenotype among blood donors in Sokoto North Western, Nigeria.

1.3. Study Population

The subjects for this study consisted of 453 consecutively-recruited blood group O donors comprising of 397 males and 56 females, aged 18 and 55 years and mean age 27.4 ± 6.6 years presenting to Usmanu Danfodiyo University Teaching Hospital (UDUTH), Specialist Hospital and National Blood Transfusion Centre, Sokoto for blood donation purpose.

1.4. Inclusion Criteria

Inclusion criteria include age ≥ 18 years, blood group O donors, residence in Sokoto State, no previous history of red cell transfusion (last 4 months) and willingness to offer verbal informed consent after pre-test counseling.

1.5. Exclusion Criteria

The following were excluded from this study; non blood donors, donors <18 years, non-blood group O donors, non-residence in Sokoto State, previous history of red cell transfusion (last 4 months) and failure to offer verbal informed consent after pre-test counseling.

1.6. Sample Collection

About two milliliter (2ml) of blood was collected from each of the study subjects and was dispensed into an EDTA anti-coagulated container for ABO and Bombay phenotype determination. All samples were run within 24 hours of collection.

2. Methodology

Testing of subject was carried out using the conventional tube method involving the Lorne Laboratories (UK) anti-H Lectin blood grouping reagent. The reagent is prepared from an extract of Ulex europaeus seeds, diluted with a sodium chloride solution containing bovine albumin. The reagent is supplied at optimal dilution for use with the tube technique. The reagent will cause agglutination (clumping) of test red cells that carry the H antigen, after centrifugation. No agglutination generally indicates the absence of the H antigen. The reagent is supplied at optimal dilution for use with the tube technique. A 3% suspension of washed test red cells was prepared in isotonic saline. One volume of % suspension of washed test red cells was added to one volume of Lorne Laboratories anti-H reagent. The mixture was mixed thoroughly and incubated at room temperature for 5 minutes. All tubes were centrifuged for 20 seconds at 1000 rpm. Red cell button was gently resuspended and read macroscopically and microscopically for agglutination.

Statistical Analysis

The data obtained from this study was analysed using Statistical Package for Social Sciences (SPSS) windows version 20 (Chicago IL). Results were expressed as means and percentages. A p-value of < 0.05 denoted a statistically significant difference in all statistical comparisons.

3. Result

Of the total 453 blood group O donors aged 18-55 years and mean age 39 ± 21 years were investigated for the presence of H-antigen using anti-H antigen. Among the

blood donors tested, we observed a 0% prevalence of Bombay phenotype. Blood donors were categorized based on ethnicity. Out of the total of 453 blood donors tested, 279 were of Hausa (61.6%), 42 were Fulani (9.3%), 48 were Hausa/Fulani (10.6%) and 84 (18.5%) were of other ethnic group. Table 2 shows the distribution of donors based on ethnicity. Gender distribution of blood donors indicated that 397 were males (87.6%) and 56 (12.4%) were females. Table 3 show the distribution of blood donors based on gender. Donors were categorized based on their age group. A significant number of blood donors 213(47.0) were in the 26-35 years age group, 111 (24.5%) were in the 36-45 years age group, 108 (23.8%) were in the 16-25 years age group and 21 (4.6%) were in the 26-55 years age group.

Table 1. *Prevalence of Bombay phenotype (O_h).*

Bombay phenotype (O_h) status	Number of Blood Donors tested	Percentage (%)
Positive	453	100
Negative	0	0
Total	453	100

Table 2. *Ethnic distribution of subjects.*

Ethnic Group	Number of Blood Donors	Percentage (%)
Hausa	279	61.6
Fulani	42	9.3
Hausa/Fulani	48	10.6
Others	84	18.5
Total	453	100

Table 3. *Gender distribution of blood donors*

Gender	Number of blood Donors	Percentage (%)
Male	397	87.6
Female	56	12.4
Total	453	100

Table 4. *Age distribution of blood donors.*

Age Group (Years)	Number of subjects	Percentage (%)
16-25	108	23.8
26-35	213	47.0
36-45	111	24.5
46-55	21	4.6
Total	453	100

4. Discussion

In this present study, we investigated a total of 453 blood group blood group O donors for Bombay phenotype. We obtained 0% prevalence among the blood donors tested. Our study is consistent with previous reports which indicate that the H-deficient Bombay phenotype is rare [4]. However, several cases of the H-deficient Bombay phenotype have been reported in other studies in India [10-11]. Also a previous report that investigated 26,638 study subjects in a tertiary care hospital in Andhra Pradesh, India showed that 13 of the subjects were Bombay phenotypes (0.048%) and that consanguinity among parents was observed in 10 cases (77%) [5]. The first known female cases of the Bombay phenotype was reported among three sisters of Bangladesh extraction [12].

Our finding is also at variance with a previous report in Tamil Nadu and Karnataka, India which indicated a Bombay phenotype prevalence of 0.004% [1]. Similarly a prevalence of 0.005% was observed among subjects in the Banglore part of India [13]. Our finding is also at variance with a previous study which reported a case of Bombay Phenotype in the Kutia Kondh Primitive Tribe of Orissa, India (14]. A more recent study [6] which investigated 836 Bhuyan subjects in the Bhuyan tribe of the Sundargarh district in North-Western Orissa, India reported three cases of the rare Bombay (Oh) phenotype. The practice of tribal and territorial endogamy, consanguinity and inbreeding among the people may be responsible for the increased homozygous expression of rare recessive genetic traits like the Bombay (Oh) phenotype.

Although the Bombay phenotype seems mostly confined to South-East Asian countries, cases have been detected in Sri Lanka[15], Thailand [16], Japan[17-18] and Malaysia [19]. Similarly, a previous report in the USA [20] reported seven cases of Bombay phenotype in two generations of an Indian family who had settled in the USA. The individuals were natives of Orissa State in India. Twenty four cases of Bombay phenotypes have also been reported [21] in Natal in South Africa among 11 unrelated Indian families who were either Tamil or Telugu speaking. Also some cases of H-deficient individuals (~1:1000) have been reported in a small French Island East of Madagascar in the Indian Ocean called the Reunion Island [21]. Several studies in different parts of India has shown varying incidence of the Bombay phenotype; 1 in 7,600 individuals in Mumbai [22], 1 in 4500 the rural population from Ratnagiri and Sindhudurg districts of Maharashtra and 1 in 18,404 amongst Indians settled in South Africa [19].

The Bombay phenotype phenomenon has a substantial clinical significance particularly in developing countries. When a patient with the Bombay phenotype need blood transfusion, they can receive only autologous blood or blood from a Bombay blood donor. A previous report [23] described a case of Bombay phenotype patient who was wrongly grouped as group O and who developed a severe haemolytic transfusion reaction after receiving group O donor blood. This observation highlights the need to carry out both forward and reverse typing in ABO blood grouping before blood is selected and cross-matched in hospital blood banks.

Similarly a rare case of severe haemolytic disease of newborn (HDN) was described in a mother with Bombay phenotype who delivered a group A baby. The father was group A and positive for H antigen [24]. Two factors which could have caused the HDFN in the baby's RBCs. Firstly, the anti A present in the mother could have been responsible for the haemolysis of the baby's red cell containing antigen A. Secondly the potent anti- H present in the Bombay phenotype

mother may have been responsible for the destruction of the H antigen containing red cells of the baby.

5. Conclusion and Recommendations

This research demonstrated a 0% prevalence of Bombay phenotype among blood group O donors in Sokoto North Western Nigeria. We recommend that all blood group O blood donors and recipient be routinely screened for Bombay phenotype to reduce the risk of a patient with Bombay phenotype being transfused blood group O blood and causing a blood transfusion reaction. There is need for the implementation of a policy on universal serum typing or reverse grouping confirmation along with 'O' cell control in reverse grouping procedure in blood transfusion laboratories in Nigeria to correlate cell and serum grouping results.

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