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RESEARCH ARTICLE

RH BLOOD GROUP SYSTEM PHENOTYPES, HAPLOTYPES AND PROBABLE GENOTYPES AMONG MAJOR SUDANESE TRIBES

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ABSTRACT

The ABO and Rh blood groups are the most important blood group systems in humans with tremendous variability among different races and ethnic groups. Their clinical importance is evident in blood transfusion and hemolytic disease of the newborn. A number of associations have been reported between diseases and blood group systems. This study aimed to determine the frequencies of Rhesus blood group phenotypes and probable genotypes of major Sudanese tribes. Following informed consent, one thousand venous blood samples were collected from unrelated individuals from ten Sudanese tribes. Red blood cells were tested for common Rhesus antigens suing Particle gel immune diffusion and slide agglutination techniques. The phenotypes, haplotypes and most probable genotypes were determined. Similarities between different Sudanese populations were calculated using Jaccard's coefficient of similarities. Phenotypic data obtained was referred to as alleles, haplotypes, genotypes based on reasonable assumptions that every Rh blood group antigen represents a gene that is always expressed and has a Mendelian dominant mode of inheritance. The $\bar{e}, \bar{C}$ and D were the most common antigens/alleles with frequencies of 98.4%, 93.8% and 90.7% respectively. The C and the E antigens/alleles were less frequent. The most prevalent haplotype complex was $\bar{c}D\bar{e}/\bar{c}\bar{d}\bar{e}$ (frequency= 44.2%) and the least common was the $CdE/\bar{c}dE$ (frequency= 0.1%).The most prevalent genotype was $\bar{c}D\bar{e}$ with a frequency of 44.2%, while $C\bar{c}D\bar{e}$ and $C\bar{c}D\bar{E}\bar{e}$ genotypes were detected with lower frequencies of 21.7% and 10.9% respectively. The $C\bar{c}DE$, $Cd\bar{e}$, CdE and $C\bar{C}E$ genotypes were the least common. In conclusion, $\bar{e}, \bar{C}$ and the D antigens/alleles were the most common among the major Sudanese tribes. The C and the E antigens were the least expressed. The Commonest Rh haplotype/genotype encountered was the $\bar{c}D\bar{e}$.

INTRODUCTION

Although there are more than 400 blood group antigens distributed among thirty blood systems in humans, the ABO and Rh are considered the major clinically significant blood group antigens. Since its discovery in the early nineteenth century, the ABO system has been shown to be highly variable among races and ethnic groups. The Rh genes code for a trans-membrane protein with about 50 antigens of which only five are clinically significant [D, C, $\bar{C}$, E, $\bar{e}$]. There are two nomenclatures for the Rh system that depend on two different theories: the Fisher-Race nomenclature postulates that there are three genes that express the Rh system antigens (C-D-E). On the other hand the Weiner theory postulates the presence of two genes, one for the D and a multi-transcribed gene that codes for (CE). In the modern days of molecular techniques, both theories have been shown to be partially correct. The Fisher-Race is widely used because it is simple to understand and use (Landsteiner, 1900; Landsteiner and Wiener, 1940; Stoia *et al*., 1967; Yamamoto, 2000; Hoffbrand and Petit, 2001; Anstee, 2009). The study of blood groups is important as it plays an important role in genetics, blood transfusion and forensic medicine (Jolly, 2000). Blood groups are believed to

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have some association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh and ABO incompatibility of the newborn (Stoia *et al.,* 1967; Skaik and El-Zyan, 2006). The Rhesus (Rh) blood group system is of clinical interest because it is involved in hemolytic disease of the newborn (HDN), hemolytic transfusion reactions and autoimmune hemolytic anemia (AIHA). Blood groups are useful genetic markers in population studies and in linkage analysis. The Rh system is easily classifiable into different phenotypes and different genotypes in different populations (Bauer, 1982).

The distribution patterns of ABO and Rh systems are complex around the world. Some variation may even occur in different areas within one small country (Kolmakova and Kononova, 1999). A significant regional heterogeneity has been reported in the (ABO) and Rh blood group gene frequencies (Kucinskas and Radikas, 1999). Moreover, one population may exhibit a high degree of similarity with a distant population that can be attributed to the common history of these populations (Mukhin, 1994). Little information is available about blood groups phenotype/genotypes frequencies among different Sudanese populations. Such information if available can be of considerable importance in clinical blood transfusion. This study aimed to determine the patterns of Rheus phenotypes, probable haplotypes/genotypes and gene complex frequencies among major Sudanese populations using gel particle immuno-diffusion techniques and Slide agglutination technique as standard. The study also aimed to determine a probable common ancestry and possible interactions between these populations.

MATERIALS AND METHODS

Study design

This was a descriptive, prospective, community-based and analytical study conducted in different parts of Sudan over two years.

Study Population

Following written informed consent 100 unrelated volunteers from each of the 10 studied tribes were bled. Tribes studied were: Galyeen, Halaween, Shaygia, Mahas and Danagla (Northern Sudan), Hadandawa (Eastern Sudan), Miseriah andZaghawa (Western tribes), Nuba and Dinka (South/West Sudan).

Blood Samples

Two and a half ml of venous blood were collected in EDTA containers.

Determination of Rh phenotypes

Rh phenotypes were determined using: Direct agglutination slide method [DiaClon, monoclonal antibodies] and the immuno-diffusion gel technique (ID-Card "DiaClon Rh – subgroups") to compare the two techniques for the first hundred samples. The remaining 900 samples were typed using the immuno-diffusion gel technique when the two techniques were found to be of similar sensitivities and specificities.

Direct agglutination slide Rh phenotypes (D, C, c, E and e) determination method

The DiaClon slide direct agglutination method is briefly as follows as per manufacturer' instructions [using Anti-CDE, Anti-C, Anti-D, anti-E, Anti-e monoclonal antibodies]:

A glass slide was identified with the patients name and tribe. The glass slide was put on heated viewing box (37^{o}C).One drop (50µL) of the respective reagent was pipettes onto the slide. One drop (50 µL) of whole blood was added. The mixture was mixed well with a clean mixing stick. While rotating the slide agglutination was observed for macroscopically. Tests were interpreted as: **positive** (antigen present)=agglutination of + [fine granular pattern] ++++ [a single clumps of red blood cells]; **negative** (antigen absent)=no visible agglutination by naked eye. The DiaMed DiaClon Rh reagents were used as part of a complete phenotype determination with "DiaClon Rhesus control".

The Immuno-diffusion Gel Technique for Rh (D, C, c, E and e) Blood grouping

The DiaMed-ID Micro Typing System utilizes a sephadex gel to capture agglutinates in a semi-solid medium. The gels column is about 75 percent packed gel and 25 percent liquid in micro tubes embedded in a plastic card to allow ease of handling, testing, reading, and disposal. The gel is a suspension of porous micro spheres whose size and distribution were selected to produce settling of non-agglutinated red cells at bottom of the micro tube and retention of agglutinate in the gel at variable levels according to their size. Retention of some red cells at the top indicates a positive result; a negative result is shown as red cells settling at the bottom. Five percent red cell is prepared in ID-Diluent as follows: 0.5 µL of ID-Diluent was dispensed into a clean tube plus 50 µL of whole blood, the cells suspension is used immediately. Known positive and negative samples are included in accordance with the relevant guidelines of quality assurance.ID-Cards "DiaClon Rh-subgroups" are identified with the individual name, tribe and number. Remove aluminum foil; add 12.5µL of the red cell suspension to all microtubes of the Card. Centrifuge the ID-Cards for 10 minutes in the ID-Centrifuge. The ID-Card is read visually and the results recorded. Results are interpreted as follows: positive= agglutinated cells forming a red line on the surface of the gel or agglutinates dispersed in gel; negative= Compact button of cells on the bottom of the micro tube.

Calculation of Jaccard's Coefficient of similarity

Then the Jaccard's similarity coefficient was use to detect the similarities between different Sudanese populations. Jaccard's coefficient was calculated using the following formula:

$$JSC = \frac{a}{a+b+c}$$

Where a is the sum of agreements (+ +), while b and c represent the sums of absent/present combinations (i.e. +/-, and _/+, respectively).

Data handling

Data was entered, checked, and analyzed using Epi info 2002 software program. The Similarity between tribes was calculated using Jaccard's similarity coefficient (JSC) formula (Jaccard, 1901).

RESULTS

The immuno-diffusion gel technique (ID-Card "DiaClon Rh – subgroups") gave identical results [sensitivity and specificity of 100%] to the slide direct agglutination test (DiaMedDiaClon Rh) for the first hundred samples tested. The rest of the samples (n=900) were only tested with the immune-diffusion gel technique. Antigen/phenotype $\bar{e}$ was found to be the most frequent in major Sudanese tribes with a high frequency of 98.4% followed by $\bar{C}$, D, C and E antigens/phenotypes with frequencies of 93.8%, 90.7%, 58.4% and 21.0% respectively (Table 1).

Tables 1. Common Rh antigens in order of frequency among Sudanese populations

Antigen	Frequency (%)
$\bar{e}$	98.4%
$\bar{C}$	93.8%
D	90.7%
C	58.4%
E	21.0%

The $\bar{e}$was the most frequent antigen among all Sudanese populations (frequencies 95-100%). The majority of the tribes (8/10; 80%) had markedly high $\bar{C}$ antigenfrequency of 91-100%. With respect to the distribution of D antigen it was observed that six tribes (6/10) had high frequency (92-99%), while the rest high had frequencies ranging from 83-88% . The prevalence of C antigen was less prevalent while the frequency of E antigen was the least common. C antigen was markedly low in the West and South West tribes [Nuba, Denka, Zagawa and Miseria (Table 2).

Tables 2. Common Rh antigens in order of frequency among major Sudanese tribes

Tribe	Rh Antigens/phenotypes Frequency (%)				
	$\bar{e}$	$\bar{C}$	D	C	E
Danagla	95	92	94	44	24
Denka	99	100	99	13	17
Halaween	100	84	98	62	22
Hadandawa	96	95	83	48	17
Gaaleen	99	82	84	56	17
Miseria	97	99	83	34	19
Mahas	100	96	88	59	36
Nuba	99	100	92	18	10
Shaigeeya	99	91	94	63	33
Zagawa	100	99	92	19	15

Phenotypically, Northern tribes were shown to be homogenous with considerable degree of similarities (65% JSC=0.7), while it was 33%, JSC0.3 between Northern and Western tribes. Similarity was perfect (100%, JSC=1) for south/south west tribes (Denka, Nuba and Zagawa). $\bar{c}D\bar{e}$ is the most prevalent phenotype complex with a frequency of 44.2%, while *CdE* and $C\bar{C}E$ were the least frequent with a frequency of 0.1%.(Table 3).

Table 3. Common phenotypes/genotypes in order of frequency among Sudanese populations

Phenotypes	Frequency (%)
$\bar{C}$ D$\bar{e}$	44.2
C $\bar{C}$ D$\bar{e}$	21.7
C $\bar{C}$ DE $\bar{e}$	10.9
CDE$\bar{e}$	6.8
C$\bar{e}$	5.7
Cd$\bar{e}$	5.6
C $\bar{C}$ $\bar{e}$	1.5
C $\bar{C}$ E$\bar{e}$	0.9
CDE	0.8
CE$\bar{e}$	0.6
CDE$\bar{e}$	0.3
CDE	0.3
C $\bar{C}$ DE	0.3
Cd$\bar{e}$	0.2
CdE	0.1
C $\bar{C}$ E	0.1

Table 4. Common haplotypes in order of frequency among Sudanese populations

Genotypes	Frequency (%)
$\bar{C}$ *D$\bar{e}$*/$\bar{C}$ *d$\bar{e}$*	44.2
CD$\bar{e}$/$\bar{C}$ *d$\bar{e}$*	21.7
CDe/$\bar{C}$ *DE*	10.9
$\bar{C}$ *DE/cd$\bar{e}$*	6.8
$\bar{C}$ *d$\bar{e}$/cd$\bar{e}$*	5.7
CD$\bar{e}$/CD$\bar{e}$	5.6
Cd$\bar{e}$/cd$\bar{e}$	1.5
Cd$\bar{e}$/cdE	0.9
$\bar{C}$ *DE/cDE*	0.8
$\bar{C}$ *dE/*$\bar{C}$ *d$\bar{e}$*	0.6
CDE/CD$\bar{e}$	0.3
CDE/CDE	0.3
CDE/ $\bar{C}$ DE	0.3
Cd$\bar{e}$/Cd$\bar{e}$	0.3
CdE/CdE	0.1
CdE/ $\bar{C}$ dE	0.1

The $\bar{c}D\bar{e}$ phenotype/genotype is highly prevalent among the Nuba and Denka with frequencies of 75 and 76% respectively, while it is less common among Shaigeeya and Mahas with frequencies of 25% and 26% respectively. The $C\bar{c}D\bar{e}$ phenotype/genotype showed low prevalence among all Sudanese tribes. The $C\bar{c}DE\bar{e}$ phenoptype/genotype had a mean frequency of 10.9% and it less than 1% in 4/10 tribes. The $\bar{c}DE\bar{e}$, $\bar{c}\bar{e}$, CDe and $C\bar{c}\bar{e}$ phenotypes/genotypes were detected with markedly low frequencies (Table 3).

The strongest similarity of gene complexes was seen between different Western tribes (75% JCS=0.8), while it was slightly lower among Northern tribes (60% JCS=0.6), low (40-50% JCS=0.5) between Eastern/Northern tribes and lowest (20% JCS=0.2) between Northern and Southern tribes. The $\bar{c}D\bar{e}/\bar{c}d\bar{e}$ (R_0r) phenotype complex was most prevalent (44.2%) among 7/10 major Sudanese populations, but not Halaween, Hadandawa and Gaaleen where the $CD\bar{e}/\bar{c}d\bar{e}$($R_1r$) is the most frequent. The $Cd\bar{e}/\bar{c}dE$ (r_yr) has the lowest frequency (0.1%) among the major Sudanese populations (Table 4).

DISCUSSION

The $\bar{e}$, $\bar{c}$ and D antigens are uniformly high among the major Sudanese tribes in contradistinction to the C and E antigens that are uniformly low. The high prevalence of D antigen was previously reported among African Americans and is different from that reported in Caucasians and native Nigerians (Neville, 1994; Jeremiah and Buscri, 2003). This is understandable, Sudanese populations like the African Americans are a mixture of Afro-Arabs and Caucasians. The high prevalence of $\bar{e}$, $\bar{c}$ and D antigens among the major Sudanese populations could suggest a common ancestry or great massive interaction some time in history. The recent separation of Sudan into Sudan and South Sudan, where more than one million individuals were relocated to South Sudan, make the latter possibility more likely. Sudan has been through multiple eras of colonization where large mass expatriation was widely practiced by the occupiers. The high prevalence of the $\bar{e}$ antigen simply reflects its wide distribution worldwide. The frequency of the $\bar{e}$ is similar to that among Nigerians living in West Nigeria but is different from those living in the East (Jeremiah and Buscri. 2003). The frequency of $\bar{c}$ and C antigens in Sudanese populations was found to be different from that reported in Nigeriansas well as Caucasians. The distribution of $\bar{c}$ antigen was highest (≥99%) in the tribes from western and southern Sudan [Denka, Nuba, Miseria and Zagawa], these tribes live in close proximity to each other with marked interactions. C antigen in the Western and Southern tribes was lower compared to northern tribes [Danagla, Mahas, Halaween, Gaaleen, Shaigeeya]. In former tribes, the Arabic roots are less than African rootsprobably suggesting a common ancestry. The E antigen was the least frequent Rhesus antigen among Sudanese population, it is markedly low among Western and southern tribes [Denka, Nuba, Zagawa, Misseria] and it is differ from that reported in Caucasians and Nigerians. The $C\bar{c}De$ phenotype/genotype was prevalent among the Halaween and Hadandawa, it was less prevalent among the Denka, Danagla and Gaaleen. The $CcDE\bar{e}$ was prevalent among northern tribes, while it was lessprevalent among Denka and Nuba. The $\bar{c}DEe$ phenotype/genotype was the reported with lower prevalences in Zagawa, Denka, Mahas, Halaween and Hadndawa. While it was lowest among Gaaleen and Nuba. The prevalences in Danagla and Shaigia are in between, probably reflecting the fact that the members of these two tribes migrated extensively to different parts of Sudan.

The $CD\bar{e}$ and $C\bar{c}\bar{e}$ Phenotypes/genotypes were not detected among Denka. $C\bar{c}\bar{e}$ phenotype/genotype was less prevalent among Hadndawa, Gaaleen, Miseria, Mahas and Nuba, it was absent among Halaween, Shaigia and Zagawa. It is reasonable to say that, although that these tribes are different, their interaction with other populations is limited. The prevalence of the $\bar{c}d\bar{e}$(r) genotype among the major Sudanese tribes was different from blacks, Caucasians (Neville, 1994). This could be attributed to the fact that the Sudanese population is a mixture of African and Arabs. In conclusion, Sudanese populations are different from Blacks and Caucasians reflecting their mixed descent. There are great similarities between tribes at different geographical locations, with some tribes showing shared characteristics due to their migratory nature.

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