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Rare Rh E/e Negative Antithetical Antigens Found Only among Women with at Least One History of Pregnancy – A Maternal Phenotype (Novel)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IZI and OE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TCA, ASI, OIA, AY and RTJ managed the analyses of the study. Authors IZI, RTJ and AY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Rh blood group antigens are hereditary characters inherited by Mendelian principle, and are useful in the population genetics study, in resolving medico-legal issues, in disease aetiology and more importantly in compatibility issues in transfusion medicine. This study was a descriptive cross-sectional study to determine the prevalence of rare Rh phenotype among patients

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requiring a red cell transfusion in a specialist hospital Sokoto.
Materials and Methods: Three millilitres of whole blood was collected from each of the patients and the red cells were screened for the presence of Rh antigens by Ortho Biovue system cassettes (AHG/Coombs) technique.
Results: In this study, the percentages of Rh D, C, c, E and e obtained were 97.3%, 24.3%, 92.2% 18.9%, and 93.6% respectively. We found no Rh null and no D deletion, however, rare Rh phenotypes were encountered among which were Rh Cc deletion and Rh E/e deletion. The Rh E/e Negative antithetical antigens encountered was 4.7% (DCc 1.0%, DC, 1.0% and Dc was 2.7%). And Cc deletion was also 4.7% (Dee, 1.3% and De, 3.4%). The Rh E/e negative phenotypes showed a statistically significant relationship with gender ($p = 0.007$), marital status ($p = 0.002$), history of pregnancy ($p < 0.001$) and ethnicity ($p = 0.028$). There was. However, no statistical significant difference found with Rh C/c negative phenotype. We also encountered no Rh e negative and Rh E/e negative among male patients, unmarried patients, Igbo patients and also Yoruba patients.
Conclusion: We conclude from this study that rare Rh E/e negative antithetical antigens are high in the study area and found only among women with at least one previous pregnancy, possibly caused by low levels of maternal hormones, suggesting a role of maternal hormones on rare Rh Ee negative antithetical phenotype.

Keywords: Rare Rh E/e negative; phenotype; Sokoto; Nigeria.

1. INTRODUCTION

Rh blood group antigens like the ABO blood group are hereditary characters inherited by Mendelian principle and are useful in the population genetics study, in resolving medico legal issues disease aetiology and more importantly in compatibility issues in transfusion medicine. About 50 antigens in the Rh system are presently known, but D, C, c, E and e are the most commonly identified and the most significant antigens in blood transfusion practice.

The Rh system is the second most clinically important after ABO blood group system because of its immunogenicity, polymorphism and complexity of its antigens. The Rh antibodies can potentially cause haemolytic transfusion reactions and haemolytic disease of the foetus and new-born. This complex system comprises of two highly homologous genes, RHD and RHCE, which encode polypeptides that are expressed on the red blood cell (RBC) surface in a protein complex. The RHD locus carries the gene for the RHD polypeptide, which expresses all the D antigen epitopes.

The RHCE locus carries the genes for the RHCE polypeptide, which expresses both the C/c and E/e antigens. The suppression of Rh antigen expression for regulator types is attributed to genetic variations, as missense point mutations, splice-site mutations, and small exonic deletions, which can affect the transcription and translation of RhAG protein that is essential for the assembly of the Rh proteins into the RBC membrane and for the integrity of RBC membranes.

The substitution for expression of the E to e polymorphism (Pro226Ala) in axon 5 of RHCE required a single nucleotide while c expression is determined by pro 103 in axon 2 [1,2]. Many variants of the e antigen have been described [2], showing that the requirements for expression of the e antigen are not fully understood. The Rh blood group antigen e is of high incidence and has many epitopes. Partial expression may occur, more commonly in black persons [3]. Rh-variant phenotypes arise through at least 4 mechanisms: (1) rearrangements of the tandemly arranged RHCE and/or RHD; (2) point mutation(s) in either gene causing amino acid change(s), with subsequent loss of some epitopes and/or expression of a low-incidence antigen; (3) nonsense mutations, and (4) deletion of nucleotides causing a frameshift and premature stop codon. For example, the presence of Val at residue 245 instead of Leu. [4,5], a deletion of Arg. at amino acid residue 229[6], or the presence of Cys (instead of Trp) at amino acid residue 16[7] affects the expression of the e antigen and occur between exons that are separated by ~30 kb[8].

Many variants recently characterised carry rearranged RH genes, most often by a unidirectional segmental DNA exchange (gene-conversion) event [8,9,10]. In D"' variants of type II, RHD is a D-CE-D hybrid gene in which the DNA fragment carrying exons 4-6 has been

replaced by the corresponding sequences from the RHCE gene [8,9,10,11,12,13].

Most rare Rh Ee negative phenotypes were case reports or accidental findings mostly on pregnant women having pregnancy complications. [1,3,14,15,16,17,18,19,20,21,22,23,24]. We intend to determine the prevalence of rare RhEe negative phenotype among patients needing a red cell transfusion.

1.1 Study Design and Area

The study was a descriptive cross-sectional study to determine the distribution of Rh C, c, E and e phenotype among 296 subjects requiring a red cell transfusion in a specialist hospital Sokoto. Sokoto is the capital city of Sokoto state, located in the extreme Northwest of Nigeria. The median age of the study population was 27.03 ± 1.035 years, about 33.1% were males while 66.9% were females and married patients were 61.1% while single was 38.9%. The sample size was computed using a software G-Power 3.0.10.

1.2 Ethical Approval and Informed Consent

Ethical clearance was obtained from the ethical committee of the Specialist hospitals, Sokoto, and written informed consent was sought from all participants in this study.

2. MATERIALS AND METHODS

Three millilitres of whole blood was collected and the red cells screened for the presence of Rh antigens by Ortho Biovue system cassettes (AHG/Coombs) technique following the manufacturer's instructions strictly.

2.1 Data Analysis

The data obtained was presented in tabular forms and in proportions and the hypothesis was tested with statistical software (SPSS version 20) at 0.05 significant levels and 95% confidence using the Person Chi-square test.

3. RESULTS

In this study, the percentages of Rh D, C, c, E and e obtained were 97.3%, 24.3%, 92.2% 18.9%, and 93.6% respectively. We found no Rh null and no D deletion, however, rare Rh phenotypes were encountered among which were Rh Cc deletion and Rh E/e deletion. The Rh E/e Negative antithetical antigens encountered was 4.7% (DCc 1.0%, DC, 1.0% and Dc was 2.7%). And Cc deletion was also 4.7% (Dee, 1.3% and De, 3.4%). The Rh E/e negative phenotypes showed a statistically significant relationship with gender ($p = 0.007$), marital status ($p = 0.002$), history of pregnancy ($p < 0.001$) and ethnicity ($p = 0.028$). There was. However, no statistical difference found with Rh C/c negative phenotype. We also encountered no Rh e negative and Rh E/e negative among male patients, unmarried patients, Igbo patients and also Yoruba patients.

4. DISCUSSION

In this study, the percentages of Rh D, C, and c were 97.3%, 24.3%, and 92.2% respectively, while the percentages for Rh E, e and E/e negative were 18.9%, 93.6% and 4.7% respectively. The prevalence of Rh E observed in our study was lower than other findings from studies done elsewhere, like the report of a study conducted earlier in Sokoto among 155 pregnant women, which reported a 28.4% [25]; 34% as previously reported among cohort of one hundred and three consecutively recruited blood donors in Aminu Kano Teaching Hospital in Kano, North- Central Nigeria [26] and also 26.55% reported among 10,133 healthy voluntary blood donors in India [27]. Our observed prevalence is however higher than that obtained in a previous study of 65 subjects made up of 35 pregnant women and 30 blood donors in the Niger Delta of Nigeria which obtained the prevalence of Rh E of 16.92% [28]. We attributed the reason for the higher value in our study compared to that done in the Niger Delta of Nigeria to the method we employed which is more sensitive and specific than the tube methods employed by the researchers who employed tube method that has a lot of errors. The other reason could be the sample size and type, while they used 65 subjects against, we used 296 patients.

Our findings of 24.9% Rh C positive closely agrees with other work done elsewhere in the same country, Nigeria [25,27,28]. Our observed prevalence is however lower than the prevalence observed in Lao population which indicate the prevalence of 60.43% [29]. Regional variation may have accounted for the difference.

The prevalence of high incident antigens Rh c and e in this study were respectively 92.2 % and 93.6%. The Rh c observed in our study is higher

Table 1. Rh E, e and E/e negative D, C and c phenotypes among the patients against gender

Parameter		Gender		Total	Pearson's Chi Square		
Rh Phenotype		**Male**	**Female**	**Total**	x^2	**df**	**p-value**
Rh E	Positive	19	37	72	0.021	1	0.885
	Negative	79	161	224			
Rh e	Positive	98	179	277	**10.049**	**1**	**0.002***
	Negative	0	19	19			
Rh_Ee deletion	Present	0	14	14	**7.273**	**1**	**0.007***
	Absent	98	184	282			
Rh D	Positive	95	193	288	0.072	1	0.789
	Negative	3	5	8			
Rh C	Positive	19	53	72	1.940	1	0.164
	Negative	79	145	224			
Rh c	Positive	93	180	273	1.455	1	0.228
	Negative	5	18	23			
Rh_Cc deletion	Present	4	10	14	0.124	1	0.725
	Absent	94	188	282			

The Rh e and E/e negative phenotypes showed a significant relationship (*) (p = 0.002 and 0.007 respectively) with gender, Rh E, C, c and Rh C/c negative however, showed no significant relationship with gender. There was no Rh e and Rh E/e negative encountered among males.

Table 2. Rh E, e and E/e negative phenotypes among the patients against ethnicity

Parameter		Ethnicity			Total	Pearson's Chi square		
Rh phenotype		**Hausa**	**Igbo**	**Yoruba**	**Total**	x^2	**df**	**p-value**
Rh E	Positive	46	9	1	56	0.712	2	0.701
	Negative	185	49	6	240			
Rh e	Positive	212	58	7	277	**9.782**	**2**	**0.008***
	Negative	19	0	0	19			
Rh_Ee deletion	Present	14	0	0	14	**7.136**	**2**	**0.028***
	Absent	217	58	7	282			
Rh_Cc deletion	Present							
	Absent							

The Rh e and E/e negative phenotypes showed a significant relationship (p = 0.008 and 0.028 respectively) with history of pregnancy, Rh E however, showed no significant relationship with marital status. There were nineteen Rh e negative and fourteen Rh E/e negative encountered among Hausa ethnic group while none was encountered among the Igbo and the Yoruba ethnic groups.

Table 3. Rh E,e and Ee negative phenotype among the patients against their marital status

Parameter		Marital Status		Total	Pearson's Chi Square		
Rh Phenotype		**Married**	**Single**	**Total**	x^2	**df**	**p-value**
Rh E	Positive	35	21	56	0.053	1	0.818
	Negative	146	94	240			
Rh e	Positive	162	115	277	**12.900**	**1**	
	Negative	19	0	19	**<0.001***		
Rh_Ee deletion	Present	14	0	14	**9.337**	**1**	**0.002***
	Absent	167	155	282			
Rh_Cc deletion	Present	7	7	14	0.799	1	0.371
	Absent	174	108	282			

The Rh e and Ee negative phenotypes showed a significant relationship (p < 0.001 and 0.002 respectively) with marital status

than that reported by Makroo and co-workers [30] who observed a prevalence of 58% among Indian blood donors and 56.3-59.7% in reported for the Indian population, and in Chinese

population of 47%. However, our finding of 92.2% is lower than the values estimated for Indian donors, Indian populations, Caucasians, Blacks and the Chinese population of respective prevalence of 98.0, 97.5-98.5, 98.0 98.0 and 96.0 percent [31,32,33].

We encountered no Rh Null or RHCE deletion in our study, this finding is contrary to the findings of Jeremiah and co-workers [34], who reported Rh D deletions among ante natal women in southern Nigerian [34]. We however, encountered some rare Rh antigens which include Rh E/e deletion (DCc, DC and Dc) of 4.7% among others. Sharma and co-workers, reported a 1.1% deletion of the antithetical antigen E/e; (0.6% cases of DCC- - and 0.5% cases of DCc- -) in Brazilian population [16] and in Nigeria, E/e negative of 0.5%, was reported among the population of cohort antenatal women in Southern Nigeria [34]. Lower figures were reported among the whites, example the -D- haplotype ranging in frequency from 0.0005 in Sweden [35] to 0.0032 in Japan [36]. We attributed the reason for the high value of Rh E/e negative antithetical antigen in our study to the consanguineous marriage practices among the Hausa and Fulani ethnic groups who are the main occupants of the area where the work was done (there was no Rh e- and Rh E/e negative encountered among Igbo and Yoruba patients as shown in Table 2).

In this study also, we do not encountered any Rh e negative and Rh E/e negative among male patients, single women or females below marital age and women with no history of pregnancy as can be seen in Tables 1, 3 and 4. There was however, a statistical significant relationship between gender and Rh e negative and Rh E/e negative ($p = 0.002$ and $p = 0.007$ respectively). This may suggest the role of female sex hormone as the main differences between males and females is the sex hormones. Sex hormones has been shown to have an influence on the number of erythrocytes in the blood, hence the difference in number in male and female blood. While the male sex hormone was said to works by an indirect effect in the kidneys by increasing the production of renal erythropoietic factor, therefore increasing the amount of active erythropoietin to act in the bone marrow; the female sexual hormones act directly on bone marrow cells and affect the production of DNA, RNA and proteins in the cells therefore affecting the rate of erythropoiesis [37]. Female sex hormones has also been reported to protect against RBC damage [38,39,40].

We also found out that Rh e negative and Rh E/e negative antithetical antigens has a statistical significant relationship with marital status ($p = < 0.001$ and 0.002 respectively) and history of pregnancy ($p = < 0.001$ and < 0.001 respectively), suggesting the role of maternal hormones. Although, gender and marriage has not been shown to have a link with gene formation, the levels maternal hormones was reported to increase during pregnancy and result to increase in haematopoietic stem-cell division, haematopoietic stem-cell frequency, cellularity, and erythropoiesis in the spleen [41]. Available literatures on Rh Ee negative, showed about 99% of the rare Rh Ee negative phenotypes were case reports or accidental findings mostly on pregnant women having pregnancy complications with pregnancy accounting for about 80% as sensitizing events [1,3,14,15, 16,17,18,19,20,21,22,23,24]. Reduction of these hormones has been shown to attenuate the increase in haematopoietic stem-cell division, haematopoietic stem-cell frequency [41], with the possibility of causing chromosomal abnormalities. This effect could be likened to the

Table 4. Rh E, e and Ee negative phenotypes among the patients against history of pregnancy

Parameter		PREG. Status		Total	Pearson's Chi Square		
Rh Phenotype		**PREG.**	**No Hist PREG.**	**Total**	**x^2**	**df**	**p-value**
Rh E	Positive	27	29	56	0.272	1	0.602
	Negative	125	115	240			
Rh e	Positive	134	143	277	**15.298**	**1**	**<0.001***
	Negative	19	0	19			
Rh_Ee	Present	14	0	14	**13.922**	**1**	**<0.001***
deletion	Absent	138	144	282			
Rh_Cc	Present	6	8	14	0.442	1	0.506
deletion	Absent	146	136	282			

The Rh e and Ee negative phenotypes showed a significant relationship ($p < 0.001$ and < 0.001 respectively) with history of pregnancy, Rh E and Rh C/c negative however, showed no significant relationship with marital status.

effect of nocodazole or monastrol drugs which tends to attenuate mitotic cell division, causing chromosomal and or DNA changes, perturb spindle organization and reduce mitotic fidelity [42,43,44,45].

Rh antigens are encoded by two genes RHD and RHCE having 10 axons each. These 10 exon genes are closely linked on the short arm of chromosome one and share about 94% sequence homology [1]. Exchange of genetic material between RHD and RHCE can result in hybrid genes which can cause weakened expression of some common Rh antigens and some rarer low-frequency Rh antigens may also be expressed e.g the RN haplotype; Lack of expression of RhCc or RhEe with normal or enhanced expression of RhD can also occur e.g –D–/–D– [1].

Sequencing results of a woman with the Rh Ee deletion suggested the presence of the hybrid allele RHCE*CE-D(4-9)-CE. Heterozygosity for RHCE*C and RHCE*c found in the woman indicates the presence of 2 RHCE alleles both lacking exons 4-9, [1] just like the RN haplotype where exon 4 of RHCE has been replaced by exon 4 of RHD and –D– haplotype where exons 2-9 of RHCE were replaced with the corresponding exons of RHD [1,8,9,10,11,12,13]. A breakpoint in introns comprising an Alu S sequence defines a recombination hot spot, leading to D-CE and CE-D transitions in this hybrid Dc- gene complex [8,9,10,11,12,13].

Expression of E and e is dependent on the Pro226Ala polymorphism present in exon 5 of RHCE. Therefore, a deletion of axon 5 will lead to the absence of the antigens [1,2]. We are therefore, insinuating, that a change in levels of the maternal hormones during pregnancy [41], among women who may have a very weak or unstable Rh e antigen causes mitotic infidelity and DNA changes possibly through a change in nucleotide, mutation, splicing, deletion or insertion in haematopoietic stem cells that produces the hybrid products [1]. Lower female hormone was reported to affects the rate of DNA synthesis leading to a programmed expression of chromosomal aberrations [46,47,48]. This was reported to occur proposedly by modulating NAD metabolism to promote NADP synthesis while inhibiting NAD breakdown to ADP ribose and nicotinamide, resulting to impair DNA synthesis and cell division [48].

Tables 1, 3 and 4 showed that there was a statistically significant relationship between phenotypes Rh e and Rh Ee with gender, marital status and history of pregnancy, but showed no statistically significant relationship between phenotypes Rh E phenotypes, this suggests that Rh e deletion might be the antigen responsible for the significant relationships and probably responsible for the effects observed in patients with total or partial RHCE gene deletion. It could also explain why the Rh e antigen is a high prevalence antigen, sometimes as high as up to 100%. It is possible that the e antigen has a very unstable and breaks off easily probably due also to its position on the RHCE gene during genomic rearrangement [49], high level of the homology (93%) and multiple recombination hot spot due to Alu S sequence [50,51,52].

5. CONCLUSION

We conclude from this study that rare Rh E/e negative antithetical antigens are high in the study area and found only among women with at least one previous pregnancy, possibly caused by low levels of maternal hormones, suggesting a role of maternal hormones on rare Rh Ee negative antithetical phenotype.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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