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Evaluation of Antinuclear Antibodies and Differential Leucocyte Count in ABO Typed Women with Spontaneous Abortion in Yenagoa, Bayelsa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to evaluate the anti-nuclear antibodies and differential leucocytes count in ABO typed women with spontaneous abortion (SA) in Yenagoa, Bayelsa State, Nigeria.
Study Design: Cohort cross-sectional study.
Place and Duration of Study: Scanex Diagnostics No 58a Nikton Road, Kpansia. Yenagoa, Bayelsa State, Nigeria, between January, 2018 and August, 2018.
Methodology: A total of 150 females were recruited; 50 with unexplained history of SA (UHSA) and 50 with unexplained recent SA (URSA) formed the test groups while 50 apparently healthy females who have children, with no recent or history of abortion were used as control. 5ml of venous blood was collected using standard venipunture technique from each participant, out of which (2mls) was dispensed into ethylene-diaminetetraacetic acid (EDTA) bottles, and was used for DLC by manual spread technique and ABO testing, using the tile method. The remaining 3ml of blood was used to analyse ANAs by the ELISA Assay technique.

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Results: The study participants, UHSAURSA and Control showed that the age group of 31-35years have the majority of spontaneous abortion of 18(36%), 13 (26%) and 12(24%) respectively. In the Blood group study, majority of blood group were B; 4 (40%), 3(22.2%), 1 (16.6%) and O 7 (22.5%), 3 (8.1%), 3 (9.6%) respectively. Out of the 9 blood group B, URSA subjects only 3 of them tested positive for ANAs, out of 10 blood group B UHSA subjects only 4 of them tested positive for ANAs & out of the 6 blood group B control participants only 1 of them tested positive for ANAs; out of the 37 blood group O URSA subjects only 3 of them tested positive for ANAs, also out of the 31 blood group O UHSA subjects, only 7 tested positive for ANAs and out of the 6 blood group O control participants only 1 tested positive for ANAs. Out of the 50 subjects with URSA, only 6 tested positive for ANAs, also out of the 50 UHSA subjects only 11 tested positive for ANAs and out of the 50 control participants only 4 tested positive for ANAs. The mean ANAs value (0.13 ± 0.11 and 0.20 ± 0.03) in URSA and UHSA were significantly lower *(p<.05*) compared to the control (0.07± 0.01) (*p=.001*) subjects. Also, the mean monocyte value (3.12±1.70and2.54±1.71) in URSA and UHSA were significantly higher *(p<.05*) when compared to the control (2.20±1.48) *(p=.020*) subjects respectively. There was no significant (p>0.05) difference between the mean neutrophil, lymphocytes and eosinophil values (54.56 ± 10.25 and 56.28 ± 11.12; 41.62 ± 11.15 and 40.56 ± 11.25; 0.32 ± 0.94 and 0.62 ± 1.01) in the same order when compared to that of the control (56.10 ± 10.56; 41.08 ± 10.58; 0.52 ± 0.88) *(p=.67; p=.89; p=.27)* subjects in the same order.

Conclusion: Age, ANAs, ABO and monocyte could be responsible for unexplained SA. It is recommended that ANAs, Blood group and DLC be adequately checked in early pregnancy, for this shall further aid the prediction and prevention of spontaneous abortion.

Keywords: Antinuclear antibodies; differential leucocyte count; ABO; women; spontaneous abortion; Yenagoa; Bayelsa State.

1. INTRODUCTION

Three or more consecutive pregnancy losses are spontaneous abortion SA [1], many causes of SA are unknown. 1% of the couple are affected [2], some SA is caused by anatomical conditions, chromosomal disorders, immune factors, ovarian factors, life style factors and infection in connection with the later disease are risk of cardiovascular complications which can be treated in some couples [3]. However, some never had a cause identified, and after extensive investigations about 50-75% of cases of SA were unexplained [2]. Spontaneous abortion can also be clinically recognized as pregnancy loss before the 20th week of gestation [4]. Spontaneous abortion is also known as miscarriage and it refers to a pregnancy that ends spontaneously before the foetus has reached a viable gestational age [5]. The World Health Organization (WHO) defines it as expulsion or extraction of an embryo or fetus weighing 500 g or less [6]. The term "foetus" will be used for most of this discussion, although the term "embryo" is the correct developmental term at ≤10 weeks of gestation. Analytical issues, risk factors and aetiology of spontaneous abortion can be reviewed and managed independently [6].

Many researches have been carried out to identify the underlying mechanisms of spontaneous abortion and evidence reveals that an immunologic mechanism is involved in some miscarriages. However, several studies on immune interactions at the feto-maternal interface and genetic-epidemiologic studies documented an immunological background for many spontaneous abortion cases [7]. Several autoantibodies, produced by the immune system directed against one or more of the individual's proteins have been investigated as possible influences on reproductive success and failure. Auto antibodies may persist for many years in the circulation as a marker of a previous autoimmune attack, but their presence does not necessarily indicate a recent disease process. The anti-phospholipid antibodies such as lupus anticoagulant, anti-cardiolipin, antinuclear, anti-β2 glycoprotein are associated with spontaneous abortion or as possible factors involved in infertility [7]. Antinuclear antibodies (ANAs), also known as an antinuclear factor (ANF) [8], are autoantibodies that bind to the contents of the cell nucleus. The immune system produces antibodies to foreign proteins (antigens) in normal individuals, but not to human proteins (auto antigens). In some individuals, antibodies to human antigens are produced [9].

Immunoglobulin G (IgG) is an antibody that is about 75% of antibodies in the serum of humans. It is mostly found in circulation [10]. The

molecules of 1gG are created and released by plasma B cells, compared to immunoglobulin M (IgM) which is the largest antibody produced by vertebrates, and it appears in response to initial exposure to antigen [11]. 1gM are responsible for the reaction caused by incompatible blood transfusion, the clumping of red blood cells if the recipient of a blood transfusion receives blood that is not compatible with their blood type [12].

White blood cells (WBCs), are cells of the immune system, they are also called leucocytes. Their role is to protect against infections and foreign bodies. White blood cells are derived from the multipotent cells of the bone marrow. WBCs are found in the blood and the lymphatic system; they all have a nucleus, differentiating them from other blood cells that do not have nuclei [13]. The amount of white blood cells in the blood shows a normal or diseased state; it is known to be a essential aspect of complete blood count.

The physiological roles of ABO blood group antigens remain a mystery despite their clinical importance. People with the common blood type O express neither the A nor B antigen, and they are perfectly healthy. Many diseases have been linked to some ABO phenotypes. For example, the ABO phenotype linked with stomach ulcers (Blood group O) and gastric cancer was linked with blood group A individuals. Another observation is that individuals with blood type O tend to have lower levels of the von Willebrand Factor (vWF), whichis a protein involved in blood clotting so these individuals could suffer from haemorrhage [14]. Shortly after the ABO blood groups were discovered, attention was directed toward the possibility of harmful effects when mother and fetus have different blood. This is the only blood group in which antibodies are constantly, predictably, and obviously present in the serum of people who lack the antigen on the red blood cells (RBCs). Spontaneous abortion is a pregnancy that ends spontaneously before the fetus has gotten to a viable gestational age [5]. ABO incompatibility occurs in 20% of pregnancies, but only 20% develop the hemolytic disease which is milder than Rh incompatibility that can lead to abortion in the uterus [15].

Spontaneous abortion (SA) is a common occurrence without predictive tools and diagnostic cause. Studies on arrays of haematological investigations that provide the necessary support to the primary activities are still not known. However in order to be able to know the pattern in diagnosing spontaneous abortion, previous studies have implicated some markers like antinuclear antibodies, differential leukocyte count and ABO blood groups, [16]. Therefore, this study was designed to identify possible markers in predicting spontaneous abortion using antinuclear antibodies (IgG), ABO antigens and immune cells of interest.

2. MATERIALS AND METHODS

2.1 Study Design

This study is a convenience cohort cross-sectional investigation carried out among women with spontaneous abortion attending emergency clinics in Yenagoa, Bayelsa State, Nigeria. The participants that were recruited for this study were grouped into two test groups; women with recent spontaneous abortion and women with a history of spontaneous abortion. Apparently healthy women who already have children with no history of spontaneous abortion constituted the control group.

2.2 Study Area

The study was carried out at the Federal Medical Centre Yenagoa, Bayelsa State, Nigeria. The hospital is the foremost tertiary health institution with referrals from all nooks and crannies of the State. A hospital with 425 bed complement and is blessed with hardworking and highly dedicated staff numbering 2,216 regular staff.Bayelsa state, is located within Latitude 4^0 15^1 North and Latitude 5^0 and 23^1 South [17]. It is also within longitude 5^0 22^1 West and 6^0 45^1 East. It is bounded by Delta State on the East, Rivers State on the West and the Atlantic Ocean on the Southern parts. Bayelsa State has eight local government area, viz: Yenagoa, Ekeremor, Brass, Nembe, Ogbia, Southern Ijaw, Sagbama, Kolokuma/Opokuma. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people [17].

2.3 Study Population

The study population was made up of one hundred and fifty (150) participants within the age range of 21-60 years consisting of 50 women with unexplained recent spontaneous abortion (URSA) and 50 women with an unexplained history of spontaneous abortion (UHSA) used as Test groups and 50 apparently healthy women who already have children with

no history of spontaneous abortion as the control group. A well-structured questionnaire was used to obtain the demographic details of the participants. Informed consent was obtained from each participant before enrollment into this study.

2.4 Eligibility Criteria

2.4.1 Inclusion Criteria

This study focused on subjects with an established history of spontaneous abortion more than two consecutive times and subjects who just had spontaneous abortion (unexplained history and recent spontaneous abortion). The control group were apparently healthy females who already had children without any history of spontaneous abortion.

2.4.2 Exclusion Criteria

Exclusion criteria include the following: subjects with history of surgeries, subjects receiving radiotherapy, subjects under long term local and systemic drug therapy, HIV- positive individuals, subjects with systemic illness, subject with failing organs, subjects that were positive to urine glucose.

2.5 Sample Size Calculation

The sample size for the study was determined using the formula:

$$N = \frac{(Z^2Pq)}{d^2} \quad [18]$$

Where:
N = Minimum number of subjects
Z = Standard Normal deviation at 95% confidence interval usually set at 1.96
q = 1 – p
d = Confidence level 0.05
P = Prevalence of spontaneous abortion incidence in Nigeria is 100.0 per 100,000 abortion cases

Therefore $\frac{100}{100,000}$ x 100
$= \frac{10}{100} = 0.10$
P = 0.10
q = 1 - 0.10
$N = \frac{(1.96)^2 \ x \ 0.10 \ x \ (1-0.10)}{(0.05)^2}$
N =138.3
= 138.

The sample size was supposed to be 138 participants but due to the difficulty in getting test subjects, a convenience sample size of 100 was used for the test group.

2.6 Sample Collection

Five millilitres (5mls) of blood was collected from each participant using standard venepuncture technique as described by [6], with a sterile disposable needle. Three millilitres (3mls) of blood was dispensed into plain bottles and, the blood in plain bottles was allowed to clot to generate serum; Pasteur pipette separated the serum for antinuclear antibodies. The separated serum was centrifuged in the laboratory using bench centrifuge at 4000rpm for two minutes to harvest clear serum. The bottles were labeled appropriately for each participant and stored in a freezer/cold store at -15^{0}C to -30^{0}C (- 20^{0}C laboratory freezer) until analyzed. The samples were thawed quickly, preferably to 8^{0}C and no warmer than room temperature. Also, 2mls of whole blood was dispensed into ethylene diamine tetraacetic acid (EDTA) bottles (di-potassium salts of EDTA, the optimal concentration is 1.5mg per ml of whole blood) for differential white blood cell count.

2.7 Laboratory Analysis

2.7.1 Determination of Antinuclear Antibodies

Method*:* Diagnostic Automation, Incorporation ANAs screen Enzyme-linked Immunosorbent Assay (ELISA) test system designed to detect IgG class antibodies was employed.

Principle: Diluted human serum was added to wells coated with purified nuclear antigens. Antinuclear Antibodies IgG specific antibody if present bound to the antigen. All unbound materials were washed away and the enzyme conjugate was added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate was washed off, and the substrate was added. The plate was incubated to allow hydrolysis of the substrate by the enzyme. The intensity of the colour is directly proportional to the amount of IgG specific antibodies in the sample.

2.7.2 ABO/ Rhesus Testing

Method: Tile Method

Principles: The ABO grouping system is based on haemagglutination reaction. When red blood cells carrying antigens are exposed to the

corresponding antibodies, they interact with each other to form a visible agglutination or clumping.

2.7.3 Methodology for Differential Leucocyte Count

Method: Manual Spread

Principles: The manual differential leukocyte count is performed to determine the relative number of each type of leukocyte present in the blood. Studies of red blood cell, white blood cell and platelet morphology are also performed. The combination of polychrome methylene blue and eosin stains has selective staining properties. The differential staining allows one to identify the types of leucocytes on the smear.

2.8 Data Analysis

A questionnaire was used to gather information from the participants. Analysis of the data obtained in the study was done using the Statistical Package for the Social Sciences (SPSS) version 22.0. Student's t-test, Analysis of variance (ANOVA), Tukey's post hoc, and Pearson Correlation tests were used to test the significance of the data. Results were presented as mean ± standard deviation and summarized as frequency (percentage). A probability value $p<.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Antinuclear antibodies were initially identified as markers to various disorders like auto immune hepatitis, systemic lupus erythematosus and sharp syndrome. However, spontaneous abortion is now known to occur when there is an underlying auto immune process that affects the development of the placenta. The association of antinuclear antibodies with spontaneous abortion has been studied by several researchers using various research methods. However, there has not been a clear cut acceptable method in tackling this problem .This probably has led to various data obtained by different studies. This work was carefully designed to study the incidence of antinuclear antibodies and differential leucocyte count in ABO typed women with spontaneous abortion, women with unexplained recent spontaneous abortion (URSA), unexplained history of spontaneous abortion (UHSA) and their values compared with apparently healthy women, who already have children with no history of abortion (control).

Table 1a. Demographic Characteristics of Study Participants

Characteristics	Overall N (%)	CONTROL N = 50 N(%)	URSA N = 50 N(%)	UHSA N = 50 N(%)
	150 (100)	50 (33.3)	50 (33.3)	50 (33.3)
Age (Mean ± SD)	33.89+7.61	35.58±8.97	33.14±7.24	32.95±6.63
Age Range				
21 – 25	24(12.6)	5(10)	9(18)	10(20)
26 – 30	28(18.6)	10(2)	10(20)	8(16)
31 – 35	43(28.6)	12(24)	13(26)	18(36)
36 – 40	24(16.0)	7(14)	8(16)	9(18)
41 – 45	15(10)	5(10)	7(14)	3(6)
46 – 50	10(6.6)	7(14)	2(4)	1(2)
51 – 55	5(3.3)	3(6)	1(2)	1(2)
56 – 60	1(2)	1(2)	0(0)	0(0)
Marital Status				
Married	150(100)	50(100)	50(100)	50(100)
Educational Qualification				
Primary School				
Secondary School	30(60)	8(26.6)	12(40)	10(33.3)
Diploma	37(74)	12(32.4)	13(35.1)	12(32.4)
Degree	24(48)	9(37.5)	7(29.1)	8(33.3)
Others	39(78)	10(25.6)	16(41.0)	13(33.3)
	20(40)	4(20)	6(30)	10(50)
Ethnic Groups				

Note: Percentages may not add up to 100 due to rounding up; frequency for each variable may vary due to non-responses or missing values, % = Percentages, N = Number of study participants, URSA= unexplained recent spontaneous abortion, UHSA= unexplained history of spontaneous abortion

Table 1b. Demographic Characteristics of Study Participants

Characteristics	Overall N (%)	CONTROL N = 50 N(%)	URSA N = 50 N(%)	UHSA N = 50 N(%)
Igbo	36(24)	0(0)	14(28)	22(44)
Hausa	7(4.6)	3(6)	4(8)	0(0)
Yoruba	17(11.3)	10(20)	2(4)	5(10)
Nembe	11(7.3)	6(34.3)	3(17.18)	2(11.4)
Epie	30(20)	6(12.6)	15(31.5)	9(18.9)
Ogbia	10(6.6)	5(31.5)	3(18.9)	2(12.6)
Ijaw	12(8)	5(26.2)	5(26.2)	2(10.5)
Ogoni	1(0.6)	0(0)	1(2)	0(0)
Kalabari	1(0.6)	0(0)	1(2)	0(0)
Ibibio	21(14)	15(30)	1(2)	5(10)
Isoko	4(2.6)	0(0)	1(2)	3(6)
Eshan	1(2)	0(0)	1(2)	0(0)
Religion				
Muslim	19(12.6)	8(16)	4(8)	7(14)
Christian	127(88)	42(84)	43(86)	42(84)
Others	4(2.6)	0(0)	3(6)	1(2)
Occupation				
Civil Servants	39(26)	16(41)	9(23)	20(51)
Housewife	5(3.3)	0(0)	2(4)	3(6)
Traders	22(14.6)	10(20)	5(10)	7(14)
Others	18(12)	10(20)	5(10)	3(6)

Note: Percentages may not add up to 100 due to rounding up; frequency for each variable may vary due to non-responses or missing values, % = Percentages, N = Number of study participants, URSA= unexplained recent spontaneous abortion, UHSA= unexplained history of spontaneous abortion.

In this study, 12% were women with unexplained recent spontaneous abortion (URSA) recorded positivity for antinuclear antibodies, 22% were antinuclear antibodies positive in women with an unexplained history of spontaneous abortion (UHSA), while only 8% of apparently healthy women were positive for antinuclear antibodies. This does not agree with the findings of Mohammed et al. [19], which established that the frequency of antinuclear antibodies was 31.8% in 110 women with history of spontaneous abortion and that only 5.7% of 35 healthy women with proven fertility and with no history of pregnancy loss or autoimmune disease, were positive. Many researchers believed that the presence of antinuclear antibodies acts as a risk factor for infertility and antinuclear antibodies can be involved in the mechanisms causing embryo implantation failure.

On the assessment of the differential leucocyte count of individuals with unexplained spontaneous abortion and controls, the results of this study showed a higher significant difference (*P=.02*) in the studied monocyte count in both unexplained recent (URSA) and history of spontaneous abortion (UHSA) when compared with the control (Table 2). This is in line with the work of Deng et al. [20], where they discovered that monocyte being the largest white blood cells, is involved in inflammation; they are more in chronic inflammation, viral infection and bacterial infection. Odutola et al. [21] also discovered that monocyte carries out phagocytic activities and they present antigen and produce cytokines. The clinical implication of low ANAs is found in chronic bacterial or viral infection and haematological malignancy patients. It is a marker for spontaneous abortion, this is because inflammation of the uterus that does not allow it to be suitable host for implantation of the embryo, natural killer cells mistake the fetal cells for cancer cells and attack them, making it unconducive for the fetus to thrive.

In this study, there is a statistically higher significant difference (*p=.02*) in antinuclear antibodies of all the participants compared to that of the controls (Table 3). ANAs were positive in some spontaneous abortion individuals and control individuals, which may be due to some underlying immune disorders. This agree with what Sarici et al. [22] reported, that ANAs are mainly associated with connective tissue diseases, and it is also found in healthy people, especially women and the elderly. Kutteh and

Odom [23] argued that spontaneous abortion reoccur when the level of antinuclear antibodies is elevated in circulation, suggesting an autoimmune disorders, which causes an immune system to attack its own cells, tissue or organ by mistake. Antinuclear antibodies are present in a patient with connective tissue disease and in healthy people according to Rai et al. [24].

This study also observed that spontaneous abortion is highest in individuals within the age range of 31 – 35years; 26% were of unexplained recent spontaneous abortion individuals and 36% were of unexplained history of spontaneous abortion individuals (Table 1b). This is similar to the findings of Nyabo et al. [25]. It was also reported that spontaneous abortion risk increases with age, especially in women aged equal or greater than 35years and that the frequency of its adverse effects in spontaneous abortion is about 10%.

Table 2. Comparison of Mean ± Standard Deviation of ANAs, Differential Leukocyte Counts of Study Participants

Parameters/Unit	URSA (a)	UHSA (b)	Control(c)	F-value	p-value	TMC
ANAs(OD RATIO)	0.13±0.11	0.20 ±0.03	0.07 ±0.01	7.747	0.001 (S)	a-c (S) b-c (S) a-b (S)
Neutrophils (%)	54.56±10.25	56.28 ± 11.12	56.10 ±10.56	0.3941	0.6750(NS)	ALL (NS)
Lymphocytes (%)	41.62±11.15	40.56±11.25	41.08±10.58	0.1161	0.8905(NS)	ALL (NS)
Eosinophils (%)	0.32 ± 0.94	0.62 ± 1.01	0.52 ± 0.88	1.3080	0.2736(NS)	ALL (NS)
Monocyte (%)	3.12 ± 1.70	2.54 ± 1.71	2.20 ± 1.48	4.020	0.020 (S)	a-c (S) b-c (S) a-b (S)

Key: NS=Non Significant; S = Significant; TMC – Total Material Consumption (defines two types of data) ALL = As per every participant

Table 3. Percentage Positivity & Negativity for Antinuclear Antibodies in the Study Population Irrespective of Blood Type

Parameters	No. of ANA Positivity	% of ANA Positivity	No. of ANAs Negativity	% of ANAs Negativity
URSA	6	12	44	88%
UHSA	11	22	39	78%
Control Subjects	4	8	46	92%

Key: ANAs = Antinuclear Antibodies

Table 4. Percentage Positivity and Negativity for Antinuclear Antibodies in Blood Group B Subjects

Parameters	No. of ANAs Positivity	% of ANAs Positivity	No. of ANAs Negativity	% of ANAs Negativity
URSA	3	22.2	47	94
UHSA	4	40	46	92
Control Subjects	1	16.6	49	98

Key: ANAs = Antinuclear Antibodies

Table 5. Percentage Positivity and Negativity for Antinuclear Antibodies in Blood Group O Subjects

Parameters	No. of ANAs Positivity	% of ANAs Positivity	No. of ANAs Negativity	% of ANAs Negativity
URSA	3	8.1	34	91.8
UHSA	7	22.5	24	77.4
Control Subjects	3	9.6	28	90.3

Key: ANAs = Antinuclear Antibodies

Table 6. Percentage Positivity and Negativity for Antinuclear Antibodies in Blood Group A& AB Subjects

Parameters	No of ANAs Positivity		% of ANAs Positivity		No of ANAs Negativity		% of ANAs Negativity	
	A	AB	A	AB	A	AB	A	AB
URSA	0	0	0	0	0	0	0	0
UHSA	0	0	0	0	0	0	0	0
Control Subjects	0	0	0	0	0	0	0	0

Key: ANAs = Antinuclear Antibodies

Table 7. Percentage of Hypersegmented Neutrophils in the Study Subjects

Parameters	No. of Hypersegmented Neutrophils	% Hypersegmented Neutrophils
URSA	18	36
UHSA	15	30
Control Subjects	6	12

Key: ANAs = Antinuclear Antibodies

In this study, hypersegmented neutrophil cells, acanthocytes, lymphocytes, schistocytes, polychromatic cells and macrocytic cells were common findings in women with spontaneous abortion (Table 2). This agrees with the study of Segolodi et al. [26], reported evidence of folate/vitamin B12 deficiency as a cause of spontaneous abortion. Hypersegmented neutrophils, macrocytes and macro ovalocytes are seen in macrocytic anaemia induced by vitamin B12 or folic acid deficiency. In the study of Yang et al. [27], it was reported that folic acid supplementation prevent megaloblastic anaemia during pregnancy and neural tube defects (NTDs) in the newborn infant. Thompson et al. [28] associated low Vitamin B12 with pregnancies affected by anencephaly (a defect in the formation of a baby's neural tube during development, baby born without parts of the brain and skull).

According to Tamura and Picciano [29], folate deficiency can result in spontaneous abortion, repeated pregnancy loss, and stillbirth. Greenberg et al. [30], in a trial from before conception to early pregnancy supplementation with folic acid, observed that folic acid decreased the risk of NTDs in fetuses. In this study there was no statistically significant relationship between spontaneous abortion and basophil, neutrophil, lymphocyte and eosinophil countsin UHSA and URSA compared to the control subjects (Table 2).

The findings of the present study are line with the results of Lucca et al. [31], who reported that normal pregnancy is accompanied by leukocytosis caused by physiological stress and that a complex physiological process increases leucocyte counts during pregnancy. According to Donbak et al. [32], the entire human population shares similar ABO and Rhesus blood groups, the frequency and distribution of the blood groups vary among nationalities and races. The distribution of these two blood groups has been repeatedly investigated in various populations all over the world and their frequencies exhibited considerable variation in different geographic locations, reflecting the underlying genetic and ethnic diversity of human populations.

Nigeria is a highly populated country comprising of different ethnic groups. As with many other genetic traits, the gene frequency of the ABO and Rh blood group varies significantly within the six geopolitical zones in Nigeria. Hassan et al. [33], reported that, it was revealed that the ABO blood group frequencies were Hassan found in the order O > A > B > AB (52.93%, 22.77%, 20.64% and 3.66%) respectively among Nigeria population. There is no direct relationship between antinuclear antibodies and the ABO blood group. However, studies have shown that a high level of antinuclear antibody test is used to measure autoimmune disorder and an autoimmune disorder causes the immune system to attack its own normal cells, tissues and organ by mistake, instead of foreign organisms. As we can see from the result above, since blood type B carries B antigen on its red cells, therefore women with URSA, UHSA & Control participants, with A blood type husbands are likely to have

tested positive for antinuclear antibodies because of autoimmune antibodies due to incompatibility. ABO incompatibility happens when a mother's blood type is O, and her baby is A or B. The mother's immune system may react and make antibodies against her baby's red blood cells.

In this study, the percentage positivity for antinuclear antibodies in blood group O subjects (Table 5). Showed 8.1% for those with URSA, 22.5% for UHSA and 9.6% for ANA in control subjects. This agrees with the study of Soni, [34], in which couples who had O type wives, A or B type husbands showed an increase in fetal loss. As far as spontaneous abortion is concerned it is higher in A-type husband and O type wife and stillbirths are higher in couples with A type husband and B-type wives, while Mohanty and Das, [35] discussed the effects of natural selection on four populations of ABO group and found that the incidence of abortion, stillbirth and post natal mortality are marginally higher among incompatible couple than a compatible couple without revealing significant statistical difference. Blood transfusion has been reported to result in high mortality before the discovery of ABO blood groups; this is because there was no knowledge of the difference in the blood composite who hadon among individuals in the human population. A later study by Landsteiner made it possible to type/group blood to ABO class (A, B and O) based on the presence or absence of surface antigens on red blood cells (RBC). The last type (AB) was discovered in 1902 by DesCasterllo and Sturli. These germane discoveries lead to reduced mortality due to blood transfusion. The human ABO type is a classic example of multi-allelism because it has three alleles (A, B and O) and its phenotype could be O, A, B or AB. The composition for an individual ABO blood type is always based on inheritance of gene on chromosome 9(9q34) which encodes glycosyl transferases that transfersomeoligosaccharides residues to H antigen, resulting in the formation of group A and B antigens but O individuals lack such activity.

4. CONCLUSION

Therefore, it is concluded that age, ANAs, ABO blood group and monocyte could be responsible for unexplained SA. It is recommended that ANAs, Blood group and DLC be adequtely checked in early pregnancy, for this shall further aid the prediction and prevention of spontaneous abortion.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office/chief editor/editorial board members of this journal. Informed consent of individual participants was sort verbally and with questionnaire. The importance of the work was pointed out to them and also how it will benefit women generally.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The ethical clearance for the study was sourced and obtained from the Ethical Committee of the Federal Medical Center, Yenagoa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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