DIFFERENTIAL LEUKOCYTE RATIOS IN WOMEN WITH UNEXPLAINED RECURRENT IMPLANTATION FAILURES

ABSTRACT

Unexplained recurrent implantation failures is currently a public health problem especially in the Sub Saharan Africa. It is a medical condition that has been attributed to many factors including low grade chronic inflammation. Differential leukocyte ratios are efficient markers of low grade chronic inflammation.The aim of the present study is to determine the levels of the differential leukocyte ratios involving the Neutrophil Lymphocyte Ratio(NLR), Monocyte Lymphocyte Ratio(MLR),Basophil Lymphocyte Ratio(BLR) and the Eosinophil Lymphocyte Ratio(ELR) in women with unexplained recurrent implantation failures and healthy controls. A total of 80 subjects involving 40 cases and 40 controls aged 20-35 years were recruited for the study.Blood samples were collected from the subjects by venipuncture.The differential leukocyte ratios were calculated manually from their fully blood count results determined with the Mindray 530BC automated analyzer, Mindray, Japan.The data was analysed with SPSS version 23 using Student test. Results was presented as Mean ± SD from the mean and $p<0.05$ considered as significant. The results revealed significant increase in the NLR(2.99 ± 0.43), ELR(4.66 ± 0.9),MLR(1.91 ± 2.06) in the women with unexplained recurrent implantation failures compared to NLR(1.80 ± 0.19), ELR(2.86 ± 0.40) and MLR(0.71 ± 1.41) respectively in the healthy women. This finding supports the claim for a low grade chronic inflammation in women with unexplained recurrent implantation failures.

Key words: Differential leukocyte ratios, Low grade chronic inflammation,unexplained recurrent reproductive failures.

INTRODUCTION

Unexplained recurrent implantation failures is defined as the repeated failures of embryos to implant in a healthy woman aged less than 40 years resulting from a failure of the embryo-endometrial immune crosstalk in at least three or more assisted reproductive therapy cycles following the transfer of competent embryos (1,2).Embryo implantation is a complex process involving apposition, adhesion and invasion of a competent embryo into a receptive endometrium within a specified period referred to as the window of implantation(3-5).It is considered the rate limiting step in assisted reproductive therapy cycles(6-9).Although advances in biomedical research has given rise to innovative strategies to improve the chances of successful implantation during assisted reproductive therapy cycles, the prevalence of unexplained recurrent implantations failures remains as high as 20%(10). In some clinics,

Comment [AA1]: (a) In the abstract subsection, there should be continuity between the background sentence and the research objective. Do not use sentences that break the continuity between the background sentences and the research objectives. Please revise/perfect it.
(b)The authors need to add the research method used (explicitly) in the Abstract section. Please add it.
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Comment [AA2]: (language) It seems that the verb ***is*** does not agree with the subject. Consider changing the verb "is" with "are"

Comment [AA3]: (language) The singular verb ***was*** does not appear to agree with the plural subject ***Results***. Consider changing the verb form for subject-verb agreement (replace "was" with "were"

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Comment [AA5]: Consider replacing the words "Key words" with "Keywords". Also note that phrases or words in Keywords must have the correct comma and one space

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patients may have to undergo as many as seven therapy cycles to achieve successful implantation(10).This is quite challenging especially for a low economic resource setting such as Nigeria considering the cost of payment for repeated therapy cycles. Differential leukocyte subsets including the neutrophils, monocytes, basophils, eosinophils and lymphocytes are associated with immune response with increase in their ratios considered an efficient marker of low grade chronic inflammations(11).There is currently a paucity of scientific data on the role of differential leukocyte ratios in Nigerian women with unexplained recurrent reproductive failures. The present study was therefore designed to determine the levels of differential leukocyte ratios in women with unexplained recurrent implantation failures compared to healthy controls.

MATERIALS AND METHODS

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Study Area

The study was conducted in Enugu State in the South East geopolitical zone of Nigeria. The state derived it's name from her capital and largest city, Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the South Eastern Nigeria. It lies between longitudes 6^o 30 'E and 6^o 55 'E and latitudes 5^o 15 'N and 7^{o}15'E. It consists of three senatorial divisions namely Enugu East, Enugu North and Enugu West (12). The ESUT Teaching Hospital is the major tertiary health facility for the State and is located at the centre of the Enugu metropolis (Parklane) for easy accessibility to Enugu residents.

Study Design

This is a cross-sectional case-controlled survey in which women with unexplained recurrent implantation failures served as the cases while age-matched healthy women with good Obstetrics and Gynecology history served as the controls. Blood samples were collected from subjects irrespective of any centre in Nigeria were she had been offered an assisted reproductive therapy services.

Ethical Considerations

Ethical clearance was obtained from the Ethical Review Committee of the Enugu State Ministry of Science and Technology, Enugu State, Nigeria.

Sample Size

The means of the differential leukocyte ratios was used to calculate a minimun sample size of 70. Group sample sizes of 35 cases and 35 controls achieved 80% power to reject the null hypothesis of zero effect size when the population effect size is 0.70(moderate to large) and the significance level(alpha) is 0.05 using a two-sided two-sample equal-variance t-test.

Subjects Recruitment

Subject selection was based on a simple random sampling procedure from a population of women with unexplained recurrent implantation failures and healthy women who gave their consent to participate in the study.

Inclusion Criteria

1. A total of 40 aged 20-35 years women who failed to achieve clinical pregnancy after a transfer of at least 3 good quality embryos in at least 3 assisted reproduction therapy cycles served as the cases.
2. A total of age-matched 40 women with good obstetrics and gynecology history served as the controls.

Exclusion Criteria

1. Women diagnosed with hematological disorders, hormonal disorders, infections, thyroid disorders, autoimmune disorders, systemic disorders such as diabetes mellitus.
2. Women with existing or previous ultrasonsgraphic evidence of uterine malformations.
3. Women with history of smoking, contraception's, alcohol or substance abuse.
4. Rhesus negative women with rhesus positive partners.
5. Women with Body Mass Index(BMI) greater than 24.99kg/m2 and/or age greater than 40 years.

Blood Sample Collection

Blood was collected from subjects using venipuncture (13).Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2-5cm just above the antecubital fossa. The antecubital fossa was cleaned using a 70% alcohol in cotton wool. A hypodermic syringe and 21G needle was inserted into the lumen of the antecubital vein and five milliliters(5ml) of blood was drawn quickly by a non traumatic pulling of the syringe piston. This was dispensed into an EDTA bottle and gently mixed.

Determination of Differential Leukocyte Ratios

The values of the Neutrophils, Basophils, Eosinophils, Monocytes and Lymphocytes were determined by the performing an automated full blood count using the Mindray 530BC automated analyzer, Mindray Japan.The samples were aspirated by letting the machine sample probe into the sample bottles and the probe button was tapped. Approximately 20ul of blood was aspirated and the values were displayed in the screen after about 30seconds as part of the full blood count results.(14).The novel differential leukocyte ratios involving the NLR, ELR,BLRand MLR were calculated manually from the values of the Neutrophil,Eosinophils, Monocyte, Basophils and Lymphocytes obtained from the full blood count results. NLR = Ratio of the neutrophil to the lymphocytes, BLR = Ratio of the Basophil count to the Lymphocyte, ELR= Ratio of the Eosinophil to Lymphocyte count while MLR = Ratio of the Monocyte to the Lymphocytes (15)

Data Analysis

Data was analysed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was defined as $p < 0.05$. Differences in the markers of platelet activation and systemic inflammation between the cases and controls was tested using t-test.

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RESULTS

The was significant difference ($p<0.05$) in the mean values of the Neutrophil to Lymphocyte Ratio(NLR), Eosinophil to Lymphocyte Ratio(ELR), and the Monocyte to Lymphocyte Ratio(MLR) between the women with unexplained recurrent implanatation failures and the healthy control women with good obstetrics and gynecology history. There was no significant differences ($p>0.05$) in the Basophil to Lymphocyte Ratio(BLR) between the women with

Comment [AA9]: (a) Each table must be accompanied by a narrative/description (explanation or description in clear and detailed words) in body text. Please provide narration/description for Table 1
(b) The author should include a graphical representation of the research results in addition to the Table.

unexplained recurrent implantation failures and the healthy controls with good obstetrics and gynecology history.

Table 1: Differential Leukocyte Ratios in the study cases and controls

Parameters	Controls (n=40)	Cases (n=40)	T-test (p-value)
NLR	1.80 ± 0.19	2.99±0.43	0.000*
ELR	2.86 ± 0.40	4.66±0.9	0.002*
MLR	0.70 ± 1.41	1.91±2.06	0.000*
BLR	0.0 ± 0.0	0.0±0.0	0.646

Key: NLR = Neutrophil to Lymphocyte Ratio, MLR= Monocyte to Lymphocyte Ratio, ELR= Eosinophil to Lymphocyte Ratio,BLR = Basophil to Lymphocyte Ratio, *significant at $p<0.05$,Data expressed as Mean±SD.

DISCUSSION

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Physiological regulation of the window of implantation has been suggested as the basis for successful implantation with perturbations in the process resulting in implantation failure. Several studies have reported significant increase in proinflammatory cytokines in women with unexplained recurrent implantation failures compared to healthy controls(16).However, the high cost and technical difficulties of evaluating inflammatory cytokines in blood samples limited the use of cytokine markers in routine clinical management of patients.The differential leukocyte ratios are cheap and easy to measure from the full blood count and have been used in the evaluation of low grade chronic inflammation in many diseases. In the present study ,the NLR,MLR and ELR were significantly increased in the women with unexplained recurrent implantation failures compared to the healthy controls. This agrees with the findings of Ashoush(17) who recorded a significant increase in the NLR in women with unexplained recurrent implantation failures compared to healthy controls. Studies have reported that the in vitro fertilization program may induce changes in some hematological parameters which in the patients which may affect the process of implantation(18,19).This may be intrinsic or caused by the hormone treatment preceding the in vitro fertilization program(20).The neutrophils, monocytes, eosinophils and basophils exerts a proinflammatory response while the lymphocytes

exerts an antinflammatory response with lymphocytopenia occurring as a result of increased apoptosis in lymphocytes. A high NLR occurs when the neutrophil count becomes high and lymphocytes low, a high MLR when the monocyte count becomes high and the lymphocyte loe, a ELR when the eosinophil count becomes high and the lymphocyte low while a high BLR ocurrs when the basophil count becomes high while the lymphocyte is low. The occurance of a low grade chronic inflammation involves an increase in the neutrophils, monocytes, basophils, eosinophils with a resultant decrease in lymphocyte counts. This observation has led to the widespread use of the NLR, MLR, ELR and BLR in the routine diagnosis and prognosis of inflammatory diseases.

The strength of the present study is that there few studies in the Nigerian population reporting the levels of the NLR, MLR, ELR and BLR in women with unexplained recurrent implantation failures compared to healthy controls. The limitation on the other hand is our small sample size as well as the non inclusion of proinflammatory cytokines such as TNF-α, IL-6 etc which have been identified with unexplained recurrent implantation failures. A study by correlating the results of the NLR, MLR, ELR and BLR with these cytokines may provide more insights into the prediction of low grade chronic inflammation in patients with unexplained recurrent implantation failures.

CONCLUSION

Significant increase in the NLR, MLR and ELR in women with unexplainened recurrent implantation failures in the present study supports the claim for low grade chronic inflammation in the etiology of unexplained recurrent implantation failures.

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Comment [AA11]: In the Conclusion subsection, the authors not only describe the research findings but also provide research novelties and suggestions for further research. Please complete it.

Comment [AA12]: Typo???

Comment [AA13]: The authors should also cite articles published by the Asian Journal of Immunology. Please cite at least 2 articles.

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