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Utility of Blood Cell Count Ratios as Biomarkers of Venous Thromboembolism Among Women on Oral Contraceptives

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ABSTRACT

Objectives: Oral contraceptives are considered reliable and safe, but there are concerns lately on the risk of venous thromboembolic complications. There is need for affordable, efficient and easy to obtain biomarkers that could be used in routine monitoring of users. The study thus considered the utility of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in assessing women on oral contraceptives for venous thromboembolic complications within the study locality.
Methods: This study was conducted at the Family Planning Unit of University of Calabar Teaching Hospital in Calabar, Cross River State of Nigeria.The blood cell counts were carried out by automation using SMART-1 Haematology Analyzer. Statistical analysis of data was done using SPSS version 22.0. A p-value of ≤ 0.05 was considered to infer a statistically significant difference.
Results: The study recorded a significantly higher (p= 0.006) value of absolute lymphocyte count among women on oral contraceptives compared to non-users. Absolute neutrophil count was significantly higher (p= 0.001), while absolute lymphocyte count was seen to be significantly lower (p= 0.001) among subjects on combined therapy. The study also observed significantly higher (p= 0.001) neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios among those who had been using combined therapy compared to those on monotherapy. **Conclusion:** The present study showed increased neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio among the study participants who were on progesterone/ estrogen combined therapy.

Key words: contraception, thrombosis, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

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INTRODUCTION

The need for proper spacing of children and overall prevention of unwanted pregnancy necessitated the integration of family planning unit as an important aspect of maternal healthcare. Among the many contraceptive options, oral contraceptive drugs are readily available and commonly used. Oral contraceptive drugs come in the form progesterone only and oestrogen-progesterone combination (1,2). While progesterone directly achieves contraception by inhibiting follicular development and preventing ovulation, oestrogen also through negative feedback on the pituitary inhibits follicular development to some extent and controls menstrual bleeding. The later exerts its action by modulating gene transcription of proteins. Unfortunately, this mechanism of action adversely extends to increased plasma concentrations of clotting factors as well, thus increasing the risk of thrombosis (3-5). The incidence of venous thromboembolic complications is generally seen to be higher in women of childbearing age for the female gender (6,7). This pattern may not be unconnected to the use of hormonal contraceptives common at this phase of life. Though oral contraceptives are considered reliable and safe, there are concerns lately on the risk of venous thromboembolic complications (8-10). To this end, there is need for affordable, efficient and easy to obtain biomarkers that could be used in routine monitoring of women on oral contraceptive drugs.

Cellular elements from peripheral blood have long been depended on for assessment of health status. Either through direct quantitative measurement of erythrocyte, leucocyte and thrombocyte counts or morphological examination of these cellular elements, they are useful in diagnosis. Changes in these parameters in association with disease occurrence and progression make them important biomarkers for laboratory evaluation in disease conditions (11,12). Beyond the information obtained by direct assessment of blood cell counts, there is an advancement in the use of full blood count components by deriving ratios of some of the conventional counts. Along this line of progress, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio have been reported to be important morbidity indicators (13). They are thought to serve as better predictors of the presence or absence of systemic coagulation disturbance. More so, since both parameters are derivatives of full blood count, their integration into routine assessment of associated disease conditions is at no additional cost. Apart from abstinence, prevention of unwanted pregnancy requires the use of one form of contraception or the other. Oral contraceptives are readily used for this purpose. However, these drugs have adverse effects including the increased risk of haematological derangement. The utility of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in assessing women on oral contraceptives for venous thromboembolic complications are yet to be evaluated within the study locality, hence the present study.

MATERIALS AND METHODS

This study was conducted among sixty-eight apparently healthy non-pregnant women of childbearing age who were accessing healthcare at the Family Planning Unit of University of Calabar Teaching Hospital in Calabar, Cross River State of Nigeria. An equal number of age-matched non-pregnant women who were not on any form of contraception served as controls. Control subjects were recruited from University of Calabar and University of Calabar Teaching

Hospital staff community. None of the study participants was on medication for at least a month prior to sample collection, while samples were collected within 9-10 am and analysed within one hour of collection to control alterations in blood count during the study period. Ethical approval was duly sought and obtained from The Ethics and Health Research Committee of University of Calabar Teaching Hospital. Informed consent was obtained from each study participant.

The blood cell counts were carried out by automation using SMART-1 Hematology Analyzer from Kinghawk Technology Co., Ltd, China. This analyser was controlled and calibrated according to manufacturer's instructions to ensure its fitness for use. A structured questionnaire was administered by three trained interviewers to obtain biodata. Student's t-test was used for comparison between two on SPSS version 22.0. Results are presented as mean and standard deviation. A p-value of ≤ 0.05 was considered to infer a statistically significant difference.

RESULTS

The study participants comprised 136 apparently healthy non-pregnant women of childbearing age. Half of this number (68) were on oral contraceptives while the remaining 68 control subjects were not on any hormonal contraception as the time of the study. Among the oral contraceptive users, 35 (51.5%) persons were on progesterone only while 33 (48.5%) others were on oestrogen-progesterone combined therapy. Duration of use for all the study participants was observed to be within one year.

Apart from significantly higher values of WBC and absolute lymphocyte count (p= 0.016, p= 0.006 respectively) among women on oral contraceptives compared to non-users, no other changes were recorded among the measured parameters (Table 1). Absolute neutrophil count was significantly higher (p= 0.001), while absolute lymphocyte count was seen to be significantly lower (p= 0.001) among subjects on combined therapy. The study also observed significantly higher (p= 0.001) neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios among those who had been using combined progesterone/estrogen contraceptives compared to those on progesterone only (Table 2).

Table 1.   **Blood cell parameters of oral contraceptive users and non-users**

Parameters	Oral Contraceptive Users n= 68	Controls (Non-users) n = 68	p-value
Age	26.13 ± 4.74	26.49 ± 5.39	0.686

HCT (l/l)	0.38 ± 0.0 4	0.37 ± 0.02	0.294
WBC (x 10^9/l)	5.24 ± 1.25	4.80 ± 0.80	0.016*
NEUT (x 10^9/l)	1.98 ± 0.93	1.88 ± 0.46	0.446
LYMPH (x 10^9/l)	2.53 ± 0.79	2.24 ± 0.33	0.006*
PLT (x 10^9/l)	208.44 ± 54.08	201.85 ± 41.98	0.429
NLR	0.91 ± 0.55	0.83 ± 0.24	0.302
PLR	90.18 ± 36.98	91.06 ± 19.63	0.862

Values are expressed as mean ± standard deviation, *Significant values.
HCT = Haematocrit, WBC = White Blood Cell Count, NEUT = Absolute Neutrophil count,
LYMPH = Absolute Lymphocyte count, PLT = Platelet count, NLR = Neutrophil-to-Lymphocyte
Ratio, PLR = Platelet-to-Lymphocyte Ratio

Table 2.   Blood cell parameters of oral contraceptive users based on drug type

Parameters	Progesterone only n = 35	Oestrogen-progesterone n = 33	p-value
Age	25.23 ± 3.40	27.09 ± 5.76	0.107
HCT (l/l)	0.37 ±0.0 4	0.38 ±0.04	0.616
WBC (x 10^9/l)	5.17 ± 1.22	5.31 ± 1.30	0.657
NEUT (x 10^9/l)	1.52 ± 0.73	2.46 ± 0.89	0.001*
LYMPH (x 10^9/l)	2.94 ± 0.76	2.08 ± 0.54	0.001*
PLT (x 10^9/l)	197.63 ± 57.24	219.91 ± 48.79	0.090
NLR	0.53 ± 0.22	1.31 ± 0.51	0.001*
PLR	70.06 ± 22.62	111.52 ± 37.52	0.001*

Values are expressed as mean ± standard deviation, *Significant values.
HCT = Haematocrit, WBC = White Blood Cell Count, NEUT = Absolute Neutrophil count,
LYMPH = Absolute Lymphocyte count, PLT = Platelet count, NLR = Neutrophil-to-Lymphocyte
Ratio, PLR = Platelet-to-Lymphocyte Ratio**.**

DISCUSSION
This research investigation assessed the utility of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as biomarkers of venous thromboembolism among women on oral contraceptives. The study recorded significantly higher values of WBC absolute lymphocyte count among women on oral contraceptives compared to non-users. Apart from this observation, no other changes were recorded among the measured parameters between oral contraceptive users and non-users. However, the observation of different oral contraceptive types among users necessitated placing them into categories for further statistical analysis. Absolute neutrophil and lymphocyte counts were seen to be significantly higher and lower

respectively among subjects on combined therapy. Furthermore, significantly elevated neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios were observed among those using combined progesterone/estrogen contraceptives compared to those on progesterone only.

Inflammatory processes can arise in thrombosis due to endothelial injuries and thrombogenesis. Systemic inflammation modifies the coagulation process and lymphocyte leading to a controlled immune response. Platelet-to lymphocyte ratio and neutrophil-to-lymphocyte ratio are thus considered as emerging practical, easily obtainable biomarkers for systemic inflammation, particularly in association with thrombosis (13-16). Under physiologic stress, the number of neutrophils increases, while the number of lymphocytes decreases. The neutrophil-to-lymphocyte ratio combines both of these changes, making it more sensitive than either alone. Platelet-to-lymphocyte ratio integrates the information about primary haemostasis and inflammation pathways and seems to be more informative than platelet count alone (17). Platelets secrete and express a large number of substances that are important mediators of coagulation, thrombosis and inflammation. Neutrophil-to-Lymphocyte ratio can be useful to rule out deep vein thrombosis when it is negative whereas Platelet-to-Lymphocyte ratio can be useful in ruling in deep vein thrombosis when positive. (16,17). The present study observed higher ratios for both parameters among those on progesterone-oestrogen combined therapy. The study participants were observed to be short-term users who had not used the oral contraceptives for more than a year. Thus, the possible impact of duration of oral contraceptives use could not be assessed. However, the implications of the current findings point towards venous thrombotic involvement, although these ratios were within their respective reference ranges.

CONCLUSSION
The present study has shown that oestrogen-containing oral contraceptives increased both neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio among the study participants (48.5%) who were on progesterone/ estrogen combined therapy.

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