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RESEARCH ARTICLE

ANTICOAGULANT DYSREGULATION IN HEAVY MENSTRUAL BLEEDING: EVIDENCE FROM ALTERED PROTEIN S AND ACTIVATED PARTIAL THROMBOPLASTIN TIME IN FEMALE UNDERGRADUATES

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Heavy Menstrual Bleeding, Activated Partial Thromboplastin Time, Protein S, Haemostasis, Anticoagulant Activity, Female Undergraduates.

Abstract

Background and Objective: Heavy menstrual bleeding (HMB) is a common gynecological condition associated with alterations in haemost atic parameters. This study aimed to evaluate haemostatic changes in female undergraduates with heavy menstrual flow by assessing Activat ed Partial Thromboplastin Time (APTT) and Protein S levels to better understand the underlying mechanisms of abnormal uterine bleeding.

Materials and Methods: A total of 80 apparently healthy female undergraduate students aged 18–26 years were recruited, comprising 40 participants with heavy menstrual bleeding and 40 with normal menstru al flow (control). Venous blood samples were collected and analyzed for APTT using the manual coagulometric (tilt-tube) method, while ProteinS levels were determined using Enzyme Linked Immunosorbent Assay (ELISA). Data were analyzed using SPSS version 22, and statisti cal significance was set at $p < 0.05$.

Results: The results showed a statistically significant increase in APTT (36.88±9.16) in participants with HMB compared to controls p=0.023(32.80±6.22; $p < 0.05$). Similarly, Protein S levels were significantly higher in the HMB group (7.03±0.97) compared to the control group p=0.001 (4.55±2.16; $p< 0.05$), indicating alterations in both coagulation and anticoagulant parameters.

Conclusion: Heavy menstrual bleeding is associated with haemostatic imbalance involving both intrinsic coagulation and anticoagulant pathways, with a more pronounced role of anticoagulant activity. These findings highlight the importance of incorporating anticoagulant markers such as Protein S in the evaluation and management of menstrual disorders.

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Introduction:-

Menstruation, which occurs roughly every 21–35 days in women of reproductive age [1-3], is a natural physiological process marked by the periodic loss of the endometrial lining of the uterus. Estrogen and progesterone levels fluctuate cyclically as a result of intricate hormonal interactions involving the hypothalamic-pituitary-ovarian axis that control the menstrual cycle. The length, frequency, and amount of blood lost during menstruation vary from person to person, although it usually lasts two to seven days [4-6].

Heavy menstrual bleeding (menorrhagia) is defined as bleeding that lasts longer than 7 days or is heavy enough to soak through pads/tampons hourly, often caused by uterine fibroids, hormonal imbalances, polyps, or IUDs. Symptoms include anemia, fatigue, and large blood clots. Treatment includes hormone therapy, NSAIDs, or surgical procedures[7]. Excessive menstrual blood loss that affects a woman's physical, social, emotional, or material quality of life is known as heavy menstrual bleeding (HMB), also known as menorrhagia [7-8]. Menstrual blood loss above 80 mL per cycle or prolonged bleeding lasting more than seven days is common clinical indicators of HMB. Frequent pad or tampon changes, including nocturnal changes, are another possible symptom. HMB is a significant part of abnormal uterine bleeding (AUB), which encompasses any variation in the frequency, duration, or volume of menstruation and may necessitate additional clinical assessment [9].

By maintaining a balance between coagulation and anticoagulation mechanisms, haemostasis plays a crucial role in controlling menstrual blood loss. The liver produces protein S, a vitamin K-dependent plasma glycoprotein that works as a cofactor for activated protein C to prevent clot formation [10–11]. Protein S is present in the bloodstream in both bound and free forms, but only the free form is functional. People with protein S deficiency are at risk of developing a type of clot called a deep vein thrombosis (DVT) that occurs in the deep veins of the arms or legs. A DVT can travel through the bloodstream and lodge in the lungs, causing a life-threatening clot called a pulmonary embolism (PE). Whether inherited or acquired, a lack of protein S has been linked to a higher risk of thrombotic diseases and may affect bleeding patterns [12]. Haemostasis is the body's rapid, multi-stage process for stopping bleeding at an injury site while maintaining normal blood flow elsewhere. It involves vascular spasm, platelet plug formation, and coagulation (clotting) to create a stable fibrin seal, followed by fibrinolysis to dissolve the clot. Key disorders include haemophilia and thrombosis. Haemostatic balance may be impacted by changes in Protein S levels brought on by physiological, inflammatory, or hormonal causes.

The APTT is a blood test, usually ranging between 25–35 seconds that measures how long it takes for blood to clot, specifically assessing the intrinsic and common pathways. It is primarily used to monitor heparin anticoagulation therapy, evaluate unexplained bleeding or bruising, and detect clotting factor deficiencies[13]. A number of coagulation factors, including factors I, II, V, VIII, IX, X, XI, and XII, are evaluated. To provide a thorough evaluation of the coagulation mechanism, APTT is frequently combined with Prothrombin Time (PT), which assesses the extrinsic route [14–16]. Anticoagulant activity is the ability of substances to prevent or inhibit blood coagulation (clotting) by interfering with clotting factors. These agents, which include medications like heparin and warfarin as well as natural compounds, typically function by inhibiting thrombin or Factor Xa, reducing fibrin formation, and slowing the clotting cascade[10].

There is little data on the connection between excessive monthly bleeding and important haemostatic markers as APTT and Protein S, especially in young female populations, despite the clinical significance of coagulation parameters in menstrual disorders. In order to increase knowledge and therapeutic management of monthly disorders, this study attempts to analyze haemostatic alterations in female undergraduates with high menstrual flow by measuring Activated Partial Thromboplastin Time and Protein S levels.

Materials and Methods:-

Study Area

The study was carried out at Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. The institution is a private tertiary institution located in the South-South geopolitical zone of Nigeria, between Owerri and Port Harcourt. Elele town is surrounded by neighboring communities namely Isikpo, Ndonii, Omagwa, Ahoada, and Omoku, making it easily accessible for research activities.

Study Population:-
A total of 80 apparently healthy female undergraduate students aged 18–26 years were recruited for this study. The participants comprised 40 female students with heavy menstrual flow and 40 female students with normal menstrual flow, who served as the control group.

Selection Criteria:-
Inclusion Criteria:-
1. Apparently healthy female undergraduate students.
2. Female students with heavy menstrual flow.
3. Female students with normal menstrual flow (control group).
4. Participants who gave informed consent.

Exclusion Criteria:-
1. Female students with any sign or symptom of unhealthiness.
2. Students with known uterine disorders such as fibroids.
3. Students with any underlying chronic diseases.
4. Students who did not give consent.

Ethical Approval/Consideration:-
Ethical approval was obtained from the Ethical and Research Committee of Madonna University Teaching Hospital, Elele, Rivers State. All participants were adequately informed about the objectives and procedures of the study, and participation was entirely voluntary.

Informed Consent:-
Written informed consent was obtained from all participants prior to sample collection. Participants who declined participation were excluded without any form of penalty.

Sample Collection:-
A standard venipuncture technique was employed to collect 5 mL of venous blood from each participant using a sterile syringe. 3ml Blood was dispensed into sodium citrate anticoagulant tubes (9:1 ration) and centrifuged at 3000 rpm for 15 minutes to obtain platelet-poor plasma for APTT analysis. The APTT was carried out on the platelet-poor plasma within 3 hours of sample collection. The plasma was analyzed within 3 hours of collection. . The 2ml of blood was dispensed into plain vacutainer tubes, allowed to clot at room temperature for 20 minutes, and centrifuged at 3000 rpm for 20 minutes for Protein S analysis. The serum was separated and stored at −20°C until analysis.

Method of Analysis:-
Protein S was determined using Enzyme Linked Immuno-Sorbent Assay (ELISA) method While Activated Partial Thromboplastin Time was determined by the manual coagulometric (tilt-tube) method.

Results:-
Data analysis was conducted using a Statistical Package for Social Science (SPSS) versions 22 Windows 10, the results were expressed in Mean±SD (standard deviation). Data was obtained from the analysis using paired samples t-test. Values were considered significant at $p<0.05$. Table 1 shows the demographic and characteristic of Heavy menstrual bleeding (HMB) and Normal menstrual bleeding (NMB) female student of Madonna University, Elele, Rivers State with mean age of 20.47±1.25 and 21.87±2.56 respectively.

Table 1: Demographic and Characteristic of Heavy menstrual bleeding (HMB) and Normal menstrual bleeding (NMB) students.

Characteristic	N	Ages (years)	Percentage (%)	Mean±SD
HMB	40	18-22	50	20.47±1.25
NMB	40	18-26	50	21.87±2.56

Table 2 shows the comparison of Activated Partial Thromboplastin Time (APTT) and Protein S between Heavy menstrual bleeding (HMB) and Normal menstrual bleeding (NMB) students using the paired samples t-test. The mean APTT in Heavy menstrual bleeding (HMB) students (36.88±9.16) was higher than in Normal menstrual bleeding (NMB) students (32.80±6.22), and this difference was statistically significant p=0.023 ($p < 0.05$). Similarly, the mean Protein S level in Heavy menstrual bleeding (HMB) students (7.03±0.97) was significantly higher than in Normal menstrual bleeding (NMB) students (4.55±2.16), with a highly statistically significant difference p=0.001 ($p < 0.05$). This suggests that both APTT and Protein S levels were significantly elevated in students with heavy menstrual bleeding compared to those with normal menstrual flow.

Table 2: Comparison of APTT and Protein S Test between Heavy menstrual bleeding (HMB) and Normal menstrual bleeding (NMB) N = 40

Parameter	HMB	NMB	t-value	p-value
APTT(Seconds)	36.88±9.16	32.80±6.22	2.371	. 0.023*
PROTEINS(ug/ml)	7.03±0.97	4.55±2.16	6.660	0.000*

Figure 1 shows Values are presented as mean ± standard deviation (SD) for Heavy Menstrual Bleeding (HMB) and Normal Menstrual Bleeding (NMB) groups (N = 40). APTT was significantly prolonged in the HMB group compared to NMB ($p = 0.023$), while Protein S levels were significantly higher in HMB ($p < 0.001$). Statistical significance was set at $p < 0.05$.

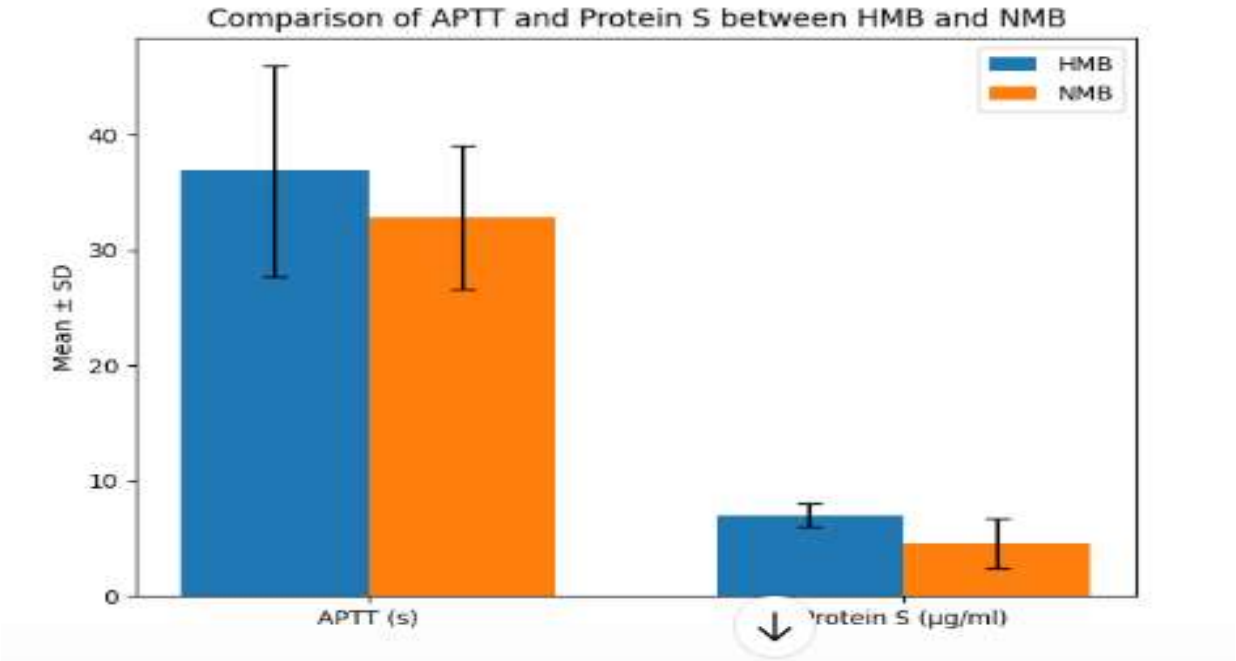

Figure 1: Comparison of Activated Partial Thromboplastin Time (APTT) and Protein S Levels between Heavy Menstrual Bleeding (HMB) and Normal Menstrual Bleeding (NMB) Groups

Discussion:-

This study evaluated haemostatic alterations in female undergraduates with heavy menstrual bleeding (HMB) by assessing Activated Partial Thromboplastin Time (APTT) and Protein S levels, providing important insights into the underlying mechanisms of menstrual blood loss. The findings demonstrated a significant increase in both APTT and Protein S levels in the HMB group compared to controls, suggesting alterations in both coagulation and anticoagulant pathways. A degree of impairment in the intrinsic coagulation system is indicated by the observed considerable extension of APTT in individuals with HMB. Factors VIII, IX, XI, and XII are among the clotting factors that APTT assesses; its prolongation may indicate minor deficits or functional inhibition within this pathway. This result is consistent with previous findings that excessive menstrual bleeding might be caused by changes in intrinsic pathway components, especially in cases linked to mild or subclinical bleeding disorders. Although statistically significant, the extension seen in this investigation might still be within physiologically normal ranges, indicating that coagulation abnormalities might not be the only factor contributing to the severity of HMB in this cohort 1-2. More strikingly, the study revealed a marked and highly significant increase in Protein S levels among participants with HMB. Protein S is a key natural anticoagulant that functions as a cofactor to activated protein C, inhibiting clot formation by inactivating factors Va and VIIIa.

The elevated Protein S levels observed may indicate an enhanced anticoagulant state, which could impair effective clot stabilization at the endometrial surface, thereby promoting prolonged or excessive bleeding. This finding supports the concept that dysregulation of anticoagulant pathways plays a critical role in the pathophysiology of HMB, even in the absence of overt coagulation factor deficiencies 10,17. A complicated haemostatic imbalance including both delayed clot formation and enhanced clot inhibition is suggested by the coexistence of prolonged APTT and high Protein S. This twofold change may result in a physiological setting where increased anticoagulant activity further impairs clot maintenance and slightly delays clot onset. The severity and duration of monthly bleeding seen in afflicted individuals may be explained by such a mechanism. This is in line with new research showing that irregular uterine bleeding is frequently complex, involving interactions between hormone control, coagulation, anticoagulation, and vascular integrity8. The results also demonstrate the drawbacks of evaluating menstruation diseases exclusively using traditional coagulation tests like APTT. The more noticeable change in Protein S highlights the significance of include anticoagulant markers in routine hemostatic testing, even though APTT demonstrated statistical significance in our investigation.

As previously noted in patients with menorrhagia without detectable coagulation abnormalities, this is especially important in situations when routine clotting tests could not adequately detect underlying hemostatic dysfunction14. The relatively homogeneous age distribution of the study population (young, apparently healthy undergraduates) minimizes confounding factors such as age-related haemostatic variability and chronic disease states. This strengthens the inference that the observed changes are likely associated with menstrual physiology and haemostatic regulation rather than underlying pathology. It also suggests that functional haemostatic alterations can occur early in life, emphasizing the need for early diagnostic evaluation in young women presenting with HMB. Clinically, these findings have important implications. The identification of elevated Protein S as a potential contributor to HMB suggests that therapeutic strategies targeting anticoagulant pathways may be beneficial. In addition, incorporating advanced haemostatic profiling into routine clinical practice could improve diagnostic accuracy and guide personalized management of menstrual disorders. However, this study has some limitations. The sample size is relatively modest, which may affect the generalizability of the findings. Additionally, other important haemostatic parameters such as Protein C, fibrinogen, D-dimer, and von Willebrand factor were not assessed. Inclusion of these markers in future studies would provide a more comprehensive understanding of the haemostatic profile in HMB.

Conclusion:-

In conclusion, this study demonstrates that heavy menstrual bleeding is associated with significant alterations in both coagulation (APTT) and anticoagulant (Protein S) pathways, with a more pronounced effect observed in the anticoagulant system. These findings suggest that both coagulation and anticoagulant pathways are involved, with a more pronounced role of anticoagulant activity in the pathophysiology of the condition. The results highlight the need for comprehensive haemostatic evaluation beyond routine tests to improve diagnosis and management of heavy menstrual bleeding.

Significance Statement:-
This study is significant as it provides novel insight into the haemostatic alterations associated with heavy menstrual bleeding, demonstrating that both coagulation and anticoagulant pathways may be involved in its pathophysiology. It highlights Protein S as an important biomarker, revealing that anticoagulant activity plays a critical role beyond what is captured by routine coagulation tests such as APTT. By exposing the limitations of conventional diagnostic approaches, the study emphasizes the need for comprehensive haemostatic evaluation in clinical practice. Furthermore, it contributes valuable population-specific data from Nigeria, addressing an important gap in African research, and offers a foundation for improved diagnostic strategies and targeted therapeutic interventions in the management of heavy menstrual bleeding.

Author Contributions:-
Kindness Emuchay and Charles Emuchay conceptualized and initiated the study. Kindness Emuchay was responsible for sample collection and data analysis. Richard Eze supervised the research work. Emmanuel Chinedu Onuoha performed the statistical analysis and prepared the manuscript draft for publication. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest:-
There is no conflict of interest.

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