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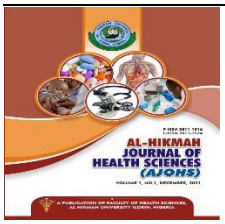

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Original Article

Impact of Gestational Age and Ethnic Origin on the Full Blood Count of Pregnant Women in Sagamu

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ABSTRACT

Pregnancy is a period in which the woman's physiology undergoes rapid changes as the baby develops into maturity and grows in size. Among the changes experienced is the haemodynamics in the pregnant mother since blood circulates and influences the development of the foetus. In this study, we investigated the blood haematological parameters of pregnant women in different stages of pregnancy progression from the first to the third trimester. Study participants included one hundred pregnant subjects that fulfilled both the inclusion and exclusion criteria for the study while forty non-pregnant women were enlisted as control. Blood sample (5 mL) was withdrawn from each participant with minimal stasis and dispensed into tubes containing Ethylene-diamine-tetra-acetic acid (EDTA) as the anticoagulant. Blood samples were then analyzed with a Coulter Analyzer. The data were presented as Mean ± SD, and statistical analysis was carried out using the student's paired t-test and ANOVA as appropriate. The result showed a significant ($p \leq 0.05$) decrease in the mean values recorded for the packed cell volume (PCV) as it decreased from 34.60±2.61 % in the first trimester to 30.83±3.27 % at the third trimester. A similar significant reduction in values were recorded for haemoglobin, MCV and MCH. There was a significant increment in the values recorded for mean MCHC across the trimesters. Our study has confirmed that pregnancy influences the decrease of many of the maternal red blood cell parameters negatively as the foetus advances in age. As a result, we suggest continuous supplementation of pregnant women with haematinics, especially as the pregnancy approaches term.

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Introduction

Pregnancy is the time during which one or more offspring(s) develops inside a woman, with the fetus being in the womb for about 40 weeks from the last menstrual period or about 38 weeks after conception. It is segmented into three trimesters of about three months each (Abman, 2011). Pregnancy nowadays can be achieved by sexual intercourse or for some others, through assisted reproductive technology. With technology, it is not uncommon to see pregnancies with multiple offsprings such as with twins (Kulkarni *et al*., 2013). Naturally, several changes occur in the physiology of the pregnant woman after conception. The health status of the pregnant woman is often a great determinant of pregnancy outcome; whereas the

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haematological indices of an individual to a large extent reflect his general health (WHO, 2011).
Many studies such as Osonuga *et al*. (2011) and Kadry *et al*. (2018) have identified the haematological indices of the pregnant woman as one of the factors influencing pregnancy. One of the best ways to detect any deviation from normal in the human body is to carry out blood tests from samples taken from the affected person in the laboratory (Nse-Abasi, *et al*., 2014). During pregnancy, the physiological alterations taking place in the woman's body would be reflected in haematological indices such as red blood cell (RBC) count, haemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Decreased counts in cell numbers are observed for example, in RBC and PLT counts caused by the physiological haemodilution that occurs in pregnancy. While it may be difficult to define a normal reference range for haemoglobin during pregnancy and the limit for diagnosing anaemia, the World Health Organization (WHO) has suggested that anaemia in pregnancies may be diagnosed when haemoglobin concentration is less than 11 g/dL. Low haemoglobin in the blood is an indication that a haematologic abnormality is present and it is often associated with adverse pregnancy outcomes (Kadry *et al*., 2018).

In uncomplicated pregnancies, there is a rise in plasma volume (PV), a situation that creates a relative or transient anaemia for the first two semesters. It is for this reason that gestational age should be taken into account when assessing haemoglobin, since the increased plasma volume causes haemodilution and leads to a decrease in plasma haemoglobin concentration (Heba *et al.*, 2018). The lower limit for haemoglobin is usually 11.5 g/dL, but for pregnant women the lower limit is usually reported as 10.0 g/dL. White blood cells, when counted along with its differentials give a picture of the body's cellular immune defense system. Since they are in circulation so as to fight infections, the counts reflect the cellular immune status of an individual at any point in time. In addition to defending the body against invasion by foreign organisms, a fraction of them partakes in the production, transportation and distribution of antibodies in immune response. An excess number of these cells in circulation therefore implies that these cells have recently been recruited to fight infections. Thus, persons with low number of blood leucocytes are exposed to high risk of disease infection, but persons having moderate counts are capable of generating antibodies and have a high degree of resistance to microbial infections (Soetan *et al*., 2013). In pregnancy, normal to moderately raised total white blood cell counts have been reported, often due to the inflammatory stress caused by pregnancy-induced physiological changes. Another category of cellular elements in blood consists of blood platelets which are involved in blood clotting. There would therefore be some delay in blood clot formation when the circulating platelet number is low, resulting in excessive loss of blood when haemorrhage has been initiated. Recent findings now suggest that platelets may be involved in body defense roles.

The counts obtained in this study were within margins of acceptable human error. Generally, laboratory data generated from sampling blood from individuals reflects to a very large extent, the input protocol starting from patient preparation to sampling and up until result is generated, although with current technology, the cellular counts obtained are more often than not, extremely accurate (Lugos *et al*., 2018). Factors that may contribute to inaccuracies include poor sample collection, storage or transportation. Storage of sample under heat or for a prolonged length of time may cause blood samples to deteriorate and affect cellular counts. Insufficient blood in a sample container may give erroneous results because of excess anticoagulant in proportion to blood. Similarly, tiny clots in blood sample may result in falsely low cellular counts (Ilesanmi, 2009).

In this study, we sought to assess the full blood count of pregnant women and the influence that gestational age and ethnic origin have on the counts.

Materials and Methods

Study Design and location

This is an observational, cross-sectional study based on blood samples of pregnant women from five public and private hospitals in Abeokuta.

Sample Population

One hundred pregnant and forty non-pregnant women (serving as control) were enlisted. Blood samples were collected from subjects that fulfill both the inclusion and exclusion criteria for the study.

Inclusion criteria

Inclusion criteria for mothers included being booked for ante-natal care and an uneventful pregnancy. Detailed history was taken from the women regarding their age, parity, gravida, socioeconomic status and physical health status.

Exclusion criteria

Exclusion criteria for mothers were: diseases complicating pregnancy (antepartum haemorrhage, pregnancy-induced hypertension, eclampsia, diabetes (gestational or insulin dependent), significant heart, kidney or lung disease, malaria, disseminated intravascular coagulation, haemoglobinopathies and

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drug or alcohol abuse. Demographic data and information on drug history were collected directly from the recruited participants, and additional data – such as HIV/hepatitis B status – were extracted from clinical notes. All study participants were on routine ferrous sulfate (200 mg three times daily), folic acid (5 mg daily), and vitamin B complex (one taken three times daily) tablets.

Ethical Approval

Enrolled pregnant women were informed about the study objective and informed consent was obtained from each. In addition, the Babcock University Research Ethical Review Board gave approval for the study.

Blood sampling

Blood sample (5 mL) was withdrawn from each participant with minimal stasis from the antecubital vein using a dry, sterile disposable syringe and needle. The blood was dispensed into tubes containing Ethylene-diamine-tetra-acetic acid (EDTA) as the anticoagulant. The specimens were labeled with the subject's age, and identification number. The EDTA samples were kept in the refrigerator and maintained at 4^{o}C until analysis, which took place not more than six hours after blood collection.

Laboratory Analysis

Full blood count using automated blood cell analyzer

The switch on the coulter analyzer was turned on and the samples' identity numbers were entered. Each sample was mixed gently but thoroughly and presented to the sample probe for aspiration and subsequent analysis. The result was displayed on the screen when the analysis was completed and the sample identity number was automatically increased by one (1) and the sample probe was replaced. The above procedure was repeated on all samples.

Statistical analysis

All calculations were done using the SPSS-V16 statistical software package for analysis of the data. The data were presented as Mean ± SD, and statistical analysis were carried out using the student's paired t-test and ANOVA as appropriate. The effects that different variables have on the full blood count of mothers were calculated using chi-square. Differences were considered to be statistically significant at an error probability of less than 0.05 ($p<0.05$).

Results

Data collected from the selected respondents were expressed as mean and standard deviation (SD), while one-way analysis of variance was used to compare the mean values while a difference of $p < 0.05$ was considered statistically significant.

Table 1: Socio Economic and demographic Characteristics of Pregnant Women

Variables	Frequency	Percentage
Age		
20-25	34	34
26-30	39	39
31-35	16	16
36-40	11	11
Mean value and SD	28.58 ±5.02	
Ethnicity		
Yoruba	87	87
Igbo	4	4
Hausa	7	7
Mean value and SD	30.15 ±3.57	
Pregnancy age		
1st trimester	15	15
2nd trimester	73	73
3rd trimester	12	12
Mean value and SD	N/A	
Number of children		
1-2	71	71
3-4	28	28
5-7	1	1
Mean value and SD	2.07±1.01	

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As shown in table 1, the pregnant women were categorized into groups with participants belonging to ages 21 – 40 years. The age bracket 26-30 had the highest frequency of 39% followed by 21-25 age group and 31-35 with frequencies of 34 and 16%, respectively; while the group having ages 36-40 had the least frequency of 11%. On gestational age, the result showed that those pregnant women in their first trimester were 15%, while the highest frequency of 73% were those in their second trimester. A minimal 12% of the pregnant women were in their third trimester. The mean value and SD for the gestational age is 4.82±1.56 months. The results showed that the majority of the respondents were Yoruba followed by Hausa with the frequencies of 87% and 9% respectively. The Igbos in the study sample were only four (4%) in number. Sagamu is a Yoruba community with fair representation of other tribes, particularly the Hausas and the Igbos. While 71% of the women have at most two children, those having more than five children constituted 1% while 28% have between three to four children with a total mean of 2.07±1.01.

Table 2: Effect of Gestational Age on Full Blood Count of Pregnant Women in Sagamu

Parameters	1st Trimester	2nd Trimester	3rd Trimester	Non-Pregnant Women	p-Value
WBC (x 10^9)	7241.67±2106.47	7793.15±1779.62	8036.67±1970.36	6945.95±1024.31	>0.05
PCV (%)	34.60±2.61	33.00±3.19	30.83±3.27	38.00±2.13	<0.05*
Haemoglobin (g/dL)	10.93±0.82	10.44±0.91	9.76±1.03	11.65±0.49	<0.01*
Platelets (x 10^9)	242.20±84.02	226.75±75.03	192.75±55.16	232.30±43.18	>0.05
MCV (fL)	86.53±5.18	85.32±7.73	83.08±11.95	86.60±6.28	<0.05*
MCH (pg)	27.39±1.77	27.03±2.71	26.35±3.99	26.70±1.78	<0.05*
MCHC (g/dL) *	31.60±0.50	31.63±0.62	31.69±0.68	31.10±0.31	<0.05*
Lymphocyte (%)	29.99±3.39	32.1±2.17	32.18±5.39	30.44±7.39	>0.05
Neutrophils (%)	54.53±5.82	60.34±4.82	61±5.02	57.92±3.82	<0.05*
Eosinophils (%)	2.91±1.44	3.34±1.04	3.01±1.23	3.68±1.11	>0.05
Monocyte (%)	3.48±1.04	3.99±1.02	4.01±1.00	3.75±1.04	>0.05
Basophil (%)	0.21±0.01	0.23±0.01	0.23±0.01	0.35±0.01	>0.05

Data presented are mean ± standard deviation, **Sig. p< 0.05**
MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; MCV= Mean cell volume; WBC=white blood cell, PCV= Packed cell volume

As presented in Table 2, the result showed varying levels of significant effect of gestational age on full blood count (FBC). Though not significant ($p>0.05$), the mean white blood cell (WBC) count increases as pregnancy advanced in age on trimester basis; with the highest value of 8036.67±1970.36 x10^9 recorded in the third trimester. The result showed a significant ($p\leq0.05$) decrease in the mean values recorded for the packed cell volume (PCV) as it decreased from 34.60±2.61 % in the first trimester to 30.83±3.27 % at the third trimester. A similar significant reduction in values were recorded for haemoglobin, MCV and MCH, however, the result obtained from the values recorded for platelets showed a non-significant effect of gestational age on the parameters though numerically, the values declined as the pregnancy advanced in age.

Also, as shown in table 2, there was a significant increment in the values recorded for mean MCHC across the trimesters with 31.60±0.50 fL recorded for first trimester and 31.69±0.68 fL for the third trimester. A similar significant increment trend was also recorded for percentage lymphocytes and neutrophils. On the contrary, the percentage eosinophil, monocyte and basophils showed no significant effect, even though the values changed numerically.

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Table 3: Effect of Ethnicity on the Full Blood Count of the Pregnant Women

Parameters	Yoruba	Igbo	Hausa	P-Value
WBC (x 10^9)	7748.85±1899.71	7325.00±1809.93	8100.00±1305.76	>0.05
PCV (%)	32.90±3.32	35.50±4.20	32.67±1.23	>0.05
Haemoglobin (g/dL)	10.39±0.96	11.40±1.42	10.39±0.36	>0.05
Platelets (x 10^9)	226.39±74.67	149.25±37.34	245.11±74.43	>0.05
MCV (fL)	85.63±8.12	84.75±5.56	81.56±7.23	>0.05
MCH (pg)	27.14±2.84	26.45±1.42	25.83±2.32	>0.05
MCHC (g/dL)	31.65±0.63	31.25±0.55	31.69±0.36	>0.05
Lymphocyte (%)	31.32±7.87	29.25±2.87	32.56±8.11	>0.05
Neutrophils (%)	61.55±7.85	64.25±2.63	61.89±7.41	>0.05
Eosinophils (%)	2.71±1.55	2.50±1.29	2.78±1.79	>0.05
Monocyte (%)	4.21±2.18	3.50±3.32	2.67±1.50	>0.05
Basophil (%)	0.33±0.52	0.50±1.00	0.11±0.33	>0.05

Data presented are mean ± standard deviation, **Sig. p< 0.05**
MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; MCV=mean cell volume; WBC=white blood cell, PCV= Packed cell volume

The statistically analyzed results for the effect of ethnicity on FBC of the pregnant women as presented in table 3 shows that the FBC parameters results were not significant ($p > 0.05$) for all the parameters observed. But numerically, it showed that Hausa tribe has the highest mean platelet value of 245.11±74.43 x10^9 followed by Yoruba and Igbo with platelet values of 226.39±74.67 x10^9 and 149.25±37.34 x10^9, respectively. In addition, the Hausa tribe has the highest mean values for WBC, MCHC, and eosinophil while the highest values for PCV, haemoglobin, basophil and neutrophils were from the Igbo tribe with data recorded showing a non-significant highest mean value for MCV, MCH and monocyte among the Yoruba tribe.

Discussion

The main aim of this study was to investigate the effect of gestational age on maternal FBC. Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are driven by hormones and the presence of the feto-placental unit. These dramatic changes influence a wide variety of haematological parameters. Acknowledgment of these changes is essential when interpreting the result of haematological investigation to diagnose or monitor illness in pregnant women, the knowledge of which will assist the healthcare provider to understand the normal physiologic and haematological changes that occur during pregnancy and thus provide a medical basis of making accurate prescription and care that is appropriate during pregnancy. As observed, there was a reduction in the PCV across the trimesters in the pregnant women as compared to non-pregnant women. The pattern of anaemia observed seems to start immediately after conception since slight anaemia, as indicated by lowered PCV, was observed even in the first trimester. It is not known what is actually responsible for the lowered haematocrit in the first trimester since volume expansion would not have become significant at this period. However, the idiosyncracies associated with early pregnancy like vomiting, nausea, anorexia may contribute to the phenomenon.

The result showed a progressive decline in haemoglobin concentration from the first to the third trimester, but a drop from first to the second trimester in pregnant women when compared with non-pregnant women. This is in spite of the ferrous sulphate supplement administered unto the subjects. The progressive decline in haemoglobin concentration from the first to third trimester may be due to an increased demand for iron as pregnancy progresses. More iron is required to meet the volume expansion of maternal haemoglobin mass and the needs of fetal growth. MCV declined from the first to third trimesters in this study, indicating gradual tendency of the RBCs to become microcytic; while MCH remained relatively stable through all trimesters. MCHC was stable in the first and second trimester but increased in the third. These findings point to a combination of iron deficiency (progressively declining MCV) and dilutional effect caused by expanded maternal blood volume (as reflected in the relatively stable MCH and MCHC but declining haemoglobin values through the various stages). Iron deficiency is one of the key factors responsible for anaemia in pregnancy.

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Gebreweld *et al*. (2018) found that 51.5% of their subjects had microcytic hypochromic anaemia; indicating iron deficiency anaemia. The high frequency of iron deficiency in pregnant women populations is because iron demand of pregnancy increases due to expanded blood volume (Heba *et al*., 2018), although the rate of iron absorption in this condition does not match the rate of utilization. It appears however that iron deficiency in most of the subjects in our study is not yet pronounced enough as to cause iron deficiency anaemia in most of them. But for the iron supplementation, the iron deficiency in most of the women would have been manifest.

The increase observed in total WBC count from the first to the third trimester in this study is consistent with the findings of Osonuga *et al*. (2011); Akinbami *et al*., (2013), and Gebreweld *et al*. (2018). The increase in leukocyte count is primarily due to an increase in neutrophil number and may represent a response to stress due to redistribution of the WBCs between the marginal and circulating pools. The gradual increase in the mean percentage neutrophil count across the semesters in this study corroborates this notion. Pain, nausea, vomiting, and anxiety have been reported to cause leukocytosis in the absence of infection (RCOG, 2006); and these may occur at the early stages of cyesis, being replaced with other stressors as the pregnancy advances. The inflammatory condition brought about by pregnancy may cause a rise in total WBC count. Furthermore, increment in WBC may be as a result of the mechanisms occurring in the maternal body that effect the building of fetal immunity, a process achieved by a state of selective immune tolerance in the presence of a strong antimicrobial immunity (Ichipi-Ifukor *et al*., 2013). In spite of the fact that the WBC is commonly increased in normal pregnancy, excessively high counts may be an indicator of haematologic malignancies. A few other conditions may also cause raised total white blood cell counts even in normal pregnancies as white blood cells in this condition respond to a variety of stimuli that cause the release of these cells from the marginal pool (Mutua *et al*., 2018). Since leukocytosis may not necessarily imply infection in the pregnant woman, laboratory results indicating raised WBCs should be interpreted with caution and further tests should be requested to confirm the diagnosis of an infection or other possible conditions in pregnant women. Both the neutrophil and lymphocyte subsets in this study show a mean percentage increase as the pregnancy advances, implying that both innate and adaptive immunity were being reinforced in the pregnant woman as the time of delivery drew near.

However, the more significant change was witnessed in the neutrophil fraction. Neutrophil counts according to Blackburn (2013) increase in pregnancy, but is not necessarily due to infection. The increase in the number of circulating neutrophils was more pronounced in the third trimester than at other stages. Inflammation becomes usually more pronounced as pregnancy approaches term; and the neutrophil fraction responds appropriately. There is no statistical difference between the value of eosinophil, monocyte and basophil in both the pregnant and non-pregnant women. This finding is similar to that of Lurie *et al*. (2008) who reported no significant increase in eosinophil count.

This study also observed a gradual reduction in platelet count as pregnancy advanced compared to non-pregnant women, which also corroborates the findings of Abbassi-Ghanavati *et al*. (2006); Ajibola *et al*. (2014) and Gebreweld *et al*. (2018). Aside anaemia, thrombocytopenia is another common hematologic abnormality that occurs during pregnancy. To be classified as having thrombocytopenia, an individual has to have a platelet count of less than $150{,}000 \times 10^9$/L (WHO). The mean values of platelets obtained in this study falls within the normal reference range for normal individuals and showed no significant difference with the non-pregnant group. Why the mean platelet count is normal across the trimesters in our study is not fully understood, but it appears that the dilutional effect occurring with RBCs is what affects platelets as well. Notwithstanding, there was reduction in mean platelet number across the trimesters up until the third trimester.

Foetal growth becomes accelerated in the third trimester, thus increasing the maternal blood volume and consequently causing a greater dilution of maternal peripheral blood cellular elements. According to Ajibola *et al*. (2014), the platelet count in normal pregnancies may decrease by approximately 10%, with most of this decrease occurring during the third trimester. It is generally believed that gestational thrombocytopenia is caused essentially by dilution of maternal plasma volume although a few workers (Eledo *et al.,* 2015) have attributed it to platelet destruction. Indeed, most workers observed only mild decreases in platelet count of pregnant women as pregnancy advances (Townsley, 2013). The mild decrease over the three semesters of gestation in the current study suggests that the lowered platelet number is not due to immune destruction; because a continuous destruction coupled with hemodilution would have resulted in severe thrombocytopenia. Bakrim *et al*. (2018) found a significant difference between the platelet count of non-pregnant women and

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the pregnant ones. They attributed gestational thrombocytopenia to platelet hyperactivity in response to a number of aggregating agents. Mutua *et al*. (2018) stated that platelet destruction in cyesis is caused by blood vessel lacerations induced by fetal expansion which activates platelet mobilization to the sites of injury. The activation and subsequent destruction then result in thrombocytopenia. Chandra *et al*. (2012) and Gebreweld *et al*. (2018) however noted that there may be pronounced thrombocytopenia in the third trimester as a result of multifactorial causes, one of which is immune-induced platelet destruction. That there is no significant thrombocytopenia among pregnant women in our study population while it obtains in some other studies suggests that there may be a geographical or genetic angle to the phenomenon.

While the results showed a varying significant effect of gestational age on the various FBC parameters tested for, the effect of ethnicity on FBC for all the parameters tested for was not significant. The wide difference in the number of participants from the Igbo and Hausa tribes compared to the Yorubas makes comparison on ethnic basis quite difficult. While genetics play a role in some blood cell production, in this case the ethnic origin does not impact the haematocrit values of the pregnant women. It appears however that diet pattern may influence red cell production in pregnancy, as our result shows a mean PCV value of 35.5% for the Igbos as compared to the Yorubas (32.9%) and Hausas (32.7%). The other red blood cell parameters also showed higher values with the Igbos compared with the other two tribes. This gap in blood values is most likely a reflection of the diet pattern among the different tribes. Although the design of this study did not factor diet pattern as a variable into the work, the Igbos are particularly known for frequent eating of vegetables, which contain several micronutrients that help not only in RBC production but also in raising the immune level of individuals.

It is already known nonetheless, that irrespective of the tribe, certain diets promote healthy red blood cell production. With regards to the platelet count, there is a wide difference in the counts obtained between the different tribes. This again supports the notion that platelet counts may be affected by racial or genetic differences among varying groups of people, especially in pregnant women. This observation requires further investigation using large sample populations for its validation. The reference range obtained in this study differs from what obtains in Caucasian populations. Differences in FBC between the African and Caucasian populations have been well-documented and are usually due to diet, genetics, level of education and social status. Beutler and West (2005)**,** established a wide variance between the FBC counts of whites and blacks in the United States of America.

Conclusion

From the current study, it is confirmed that pregnancy in women alters haematological indices such as PCV, haemoglobin, MCV, and a number of other blood parameters either negatively or positively as the pregnancy advances. In addition, ethnicity among Nigerians in the study site showed no effect on haematological parameters.

Recommendation

It is recommended that nutritional supplementation during pregnancy should be mandated for pregnant women in the study region so as to make up for any nutritional deficiency that may be present during this period; since pregnancy by its nature predisposes the woman to different forms of deficiencies that affect the FBC. This is even more so for those in the advanced stage who should be given supplements that boost RBC production, since gestational age affects both PCV and haemoglobin levels.

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