

## Effect of Time of Sample Collection on Some Haematological Parameters in Nnewi, Anambra State, Nigeria

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### Abstract

**Background:** Laboratory analysis and the results outcome play an important role in patient well-being, and the time of the day when blood sample is collected for laboratory analysis have effect on laboratory parameters results. This may lead to wrong interpretation of the result as well as wrong diagnosis. The times of the day are morning, afternoon and evening. The aim of the study therefore was to study the effect of time of sample collection for analysis on some haematological parameters in Nnewi Anambra state, Nigeria.

**Methodology:** Whole blood samples were collected from 400 adults (male and female) between 9:00 am-10:30 am in the morning, and collection was repeated in the afternoon (1:00 pm-2:30 pm), and the evening (5:00 pm-6:30 pm) from the same willing adult subjects. Hematological analyses were carried out on the blood samples immediately upon collection. Kruskal Wallis statistical tool was used for the analysis, using SPSS version 21 software package.

**Results:** Significant differences ( $p=0.00$ ) were observed in the different time of the day (morning, afternoon and evening) for erythrocyte sedimentation rate (ESR) values, Red blood cell (RBC) values, white blood cell (WBC) values and the mean cell haemoglobin (MCH) values. No significant differences ( $p>0.05$ ) were seen in the values of Haemoglobin (HB), Packed Cell Volume (PCV), Mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), and platelet in morning, afternoon and evening.

**Conclusion:** Period of blood collection had effect on erythrocyte sedimentation rate, red blood cell, white blood cell values and the mean cell haemoglobin values. But no effect on other haematology parameters was studied.

**Keywords:** Haematological values; Morning; Afternoon; Evening

### Introduction

Laboratory tests are ordered on a daily basis when patient visit the hospital at any time of the day [1]. The time of day are divides into 3 categories; morning, afternoon and evening. Morning is from 12:01 am to 12:00 noon. Afternoon is from 12:01 pm to around 5:00 pm. Evening is from 5:01 pm to 8:00 pm [2]. Many laboratory parameters vary depending on the time of day, week, month or year when they are sampled [3]. To allow for this effect, some laboratory tests are recommended at specific times of the day, e.g. testosterone should be sampled between 7 am-10 am. This is because peak testosterone levels usually occur in the early morning; evening levels are often substantially (up to 50%) lower, especially in younger males [3]. Serum cortisol are preferably be sampled in the early morning as there is marked diurnal variation, with early morning levels at least 50%-100% higher than levels in the late afternoon [4] Previous studies have shown that some biochemical components exhibit diurnal variation and/or are sensitive to postural change [5]. Diurnal variation of iron is well documented published studies offer conflicting conclusions about the pattern of variation. The prevailing opinion is that iron levels are higher in the morning than in the afternoon or evening [6]. However, some studies have shown iron levels to be higher in the afternoon or evening than in the morning, and other studies have found significant variation but no systematic trend [7] Erythrocyte Sedimentation Rate (ESR) is a blood test that detects and monitors inflammation in the body. It measures the rate at which Red Blood Cells (RBCs) in a test tube separate from blood serum over time, becoming sediment in the bottom of the test tube. The sedimentation rate increases with more inflammation, and also a rise in temperature [8]. White cell total count is part of the complete blood count. It is helpful in the evaluation of infection,

allergy, neoplasm or immunosuppression [9]. Eating, physical activity and stress may increase the WBC count and alter the differential values. WBC count tends to be lower in the morning and higher in the late afternoon [10]. Nnamdi Azikiwe University Teaching hospital runs 24 hours laboratory services, blood samples are collected at any time for laboratory analysis in Nnewi. Nnewi is the second largest city in Anambra State in southeastern Nigeria. The question is can one obtain the same laboratory result at any time of the day the blood sample is collected for some haematological parameters of well-made and stained blood films.

### Methodology

Apparently healthy adults both male and female aged 20 to 45-years-old, totaling 400 were used for this study. Whole blood samples were collected in the morning between 9:00 am-10:30 am, and collection was repeated in the afternoon (1:00 pm-2:30 pm), and the evening (5:00 pm-6:30 pm) from the same willing adults. Two milliliters (2 ml) syringe was used for the collection of blood from the cubital vein of the arm which

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appears prominent from each individual during each sample collection, and dispensed into di-potassium ethylenediamine tetra acetic acid (K2 EDTA) container, for Full Blood Count (FBC). The parameters analyzed were; Haemoglobin (Hb) in g/dl, Packed Cell Volume (PCV) in %, White blood cell count (WBC) in  $\times 10^6/l$ , Red blood cell count (RBC) in  $\times 10^{12}/l$ , Mean Cell Haemoglobin (MCH) in pg, Mean Cell Haemoglobin Concentration (MCHC) in %, Mean Cell Volume (MCV) in, Platelet count in  $\times 10^6/l$  were done using Haematology analyzer (Erma PCE 210 made in Japan). And also Erythrocyte sedimentation rate (ESR) in mm/hr was measured using Westergren method. The data collected were analyzed using SPSS software package (version 21.0) for Windows. The results were presented as mean  $\pm$  Standard Deviation (SD). Level of significant (P value  $\leq$  0.05) and confidence interval of 95% were considered as statistically significant at that level. Kruskal Wallis was used to compare variables.

## Results

The ESR shows significant different ( $p=0.00$ ) between morning time and afternoon time between afternoon time and evening time of sample collection. The value of ESR in the afternoon was significantly higher, when compared with the values obtained in the morning and the evening hours. The mean  $\pm$  SD ESR (mm/hr) were  $11.55 \pm 4.76$  in the morning,  $18.32 \pm 4.6$  mm/hr the afternoon,  $11.17 \pm 2.11$  mm/hr in the evening. The mean  $\pm$  SD WBC ( $\times 10^9/l$ ) were  $4.41 \pm 0.94$  ( $\times 10^9/l$ ) in the morning,  $5.57 \pm 1.12$  ( $\times 10^9/l$ ) in the afternoon,  $4.36 \pm 0.73$  ( $\times 10^9/l$ ) in the evening. There were significant differences in the means ( $P=0.00$ )

(Table 1a). WBC showed significant differences in the different time of blood sample collection (morning, afternoon and evening). The value obtained in the afternoon was significantly higher when compared with the values in the morning and evening. The mean  $\pm$  SD of red blood cells (RBC) were  $5.75 \pm 0.94$  ( $\times 10^{12}/l$ ) in the morning,  $5.80 \pm 0.58$  ( $\times 10^{12}/l$ ) in the afternoon,  $4.56 \pm 0.57$  ( $\times 10^{12}/l$ ) in the evening. There were significant differences in the means ( $P=0.00$ ) (Table 1b). The value of RBC count was higher in the morning and the afternoon when compared with the values obtained in evening. There were significant differences in the means of MCH and MCV in the morning, afternoon and evening. Haemoglobin, Packed Cell Volume (PCV), Platelet and MCHC values showed no significant difference in different time of blood samples collections as shown in the Tables 1a, 1b and 1c.

## Discussion

Laboratory tests help to determine the presence, extent or absence of disease and monitor the effectiveness of treatment. An estimated 60 to 70 percent of all decisions regarding a patient's diagnosis, treatment, hospital admission and discharge are based on laboratory test results. Unreliable performance can result in misdiagnosis, delayed treatment and increased costs due to retesting. It is therefore of great importance to ensure all results provided are both accurate and reliable, one of the factors that affect these, is the time of blood sample collection. From the result, Erythrocyte sedimentation values were higher in the afternoon when compared with the values obtained in the morning and evening. The differences were seen only among the female subject. This can be

Haematological parameters	Periods of blood collection			p-values
	Morning 9:00-10:30 am (n=400)	Afternoon 1:00-2:30 pm (n=400)	Evening 5:00-6:00 pm (n=400)	
ESR (mm/hr)	$11.55 \pm 4.76$	$18.32 \pm 4.63$ d,f	$11.17 \pm 2.11$	0.00*
HB (g/dl)	$12.30 \pm 1.18$	$36.37 \pm 3.33$	$36.45 \pm 3.21$	0.95
PCV (%)	$36.44 \pm 38.4$	36-33	$44 \pm 3.39$	0.01*
WBC ( $\times 10^9/l$ )	$4.41 \pm 0.94$	$5.57 \pm 1.12$ d	$4.36 \pm 0.73$ .	0.12
RBC ( $\times 10^{12}/l$ )	$5.75 \pm 0.94$	$5.80 \pm 0.58$	$4.56 \pm 0.57$ e	0.03
PLATELET	$192.12 \pm 30.75$	$193.66 \pm 30.22$	$189.44 \pm 21.77$	0.09
MCH (pg)	$22.74 \pm 2.22$	28.45	$23.08 \pm 2.33$ e	0.04*
MCHC (%)	$33.48 \pm 2.20$	$33.32 \pm 2.05$	$33.06 \pm 2.24$	0.06
MCV (fl)	$64.88 \pm 4.69$	$64.89 \pm 2.29$	$65.89 \pm 4.71$ e	0.01*

Data is expressed as mean  $\pm$  SD; \* Significant ( $P \leq 0.05$ )  
d=Morning (9:00-10:30 am) compared to Afternoon (1:00- 2:30 pm)  
e=Morning (9:00-10:30 am) compared to Evening (5:00-6:00 pm)  
f =Afternoon (1:00-2:30 pm) compared to Evening (5:00-6:00 pm)

**Table 1a:** Comparison of ESR, HB, PCV, WBC, RBC, platelet and RBC indices (MCH, MCHC, MCV) at different times of sample collection)

Haematological parameters	Periods of blood collection			P-values
	Morning 9:00-10:30 am (n=200)	Afternoon 1:00-2:30 pm (n=200)	Evening 5:00-6:00 pm (n=200)	
ESR (mm/hr)	$9.90 \pm 3.90$	$10.76 \pm 3.85$	$9.07 \pm 2.43$	0.3
HB (g/dl)	$12.87 \pm 1.33$	$12.83 \pm 1.31$	$12.84 \pm 1.20$	0.66
PCV (%)	$37.68 \pm 3.69$	$37.38 \pm 3.72$	$37.44 \pm 3.64$	0.95
WBC ( $\times 10^9/l$ )	$37.68 \pm 3.69$	$5.75 \pm 1.31$	$4.37 \pm 0.90$ e,f	0.00*
RBC ( $\times 10^{12}/l$ )	$5.86 \pm 0.67$	$6.83 \pm 0.61$	$5.67 \pm 0.67$ e,f	0.00*
PLATELET	$187.51 \pm 26.46$	$191.48 \pm 28.66$	$188.87 \pm 21.64$	0.09
MCH (pg)	$187.51 \pm 26.46$	$22.73 \pm 2.70$	$23.62 \pm 2.85$	0.31
MCHC (%)	$33.23 \pm 2.18$	$33.25 \pm 1.93$	$33.20 \pm 2.20$	0.12
MCV (fl)	$63.53 \pm 4.68$	$63.39 \pm 4.26$	$65.17 \pm 4.72$ e,f	0.00*

Data is expressed as mean  $\pm$  SD; \* Significant ( $P \leq 0.05$ )  
d=Morning (9:00-10:30 am) compared to Afternoon (1:00-2:30 pm)  
e=Morning (9:00-10:30 am) compared to Evening (5:00-6:00 pm)  
f=Afternoon (1:00-2:30 pm) compared to Evening (5:00-6:00 pm)

**Table 1(b):** Comparison of ESR, HB, PCV, WBC, RBC, platelet and RBC indicates (MCH, MCHC, MCV) of the male subject at different time of sample collection

Haematological parameters	Periods of Blood Collection			P-value
	Morning 9:00-10:30 am n=200	Afternoon 1:00-2:30 pm n=200	Evening 5:00-6:00 pm n=200	
ESR (mm/hr)	12.10 ± 5.52	15.90 ± 4.91 <sup>d,f</sup>	11.25 ± 3.57	0.00*
HB (g/dl)	11.92 ± 0.93	11.94 ± 0.97	11.81 ± 0.91	0.05
PCV (%)	35.28 ± 2.59	35.36 ± 2.51	35.37 ± 2.49	0.65
WBC (× 10 <sup>9</sup> /l)	4.25 ± 0.83	4.33 ± 0.85	4.20 ± 0.67	0.24
RBC (× 10 <sup>12</sup> /l)	5.63 ± 0.63	5.64 ± 0.54	4.58 ± 0.51 <sup>e,f</sup>	0.03*
PLATELET	196.56 ± 33.74	195.87 ± 31.67	195.08 ± 21.94	0.08
MCH (pg)	22.75 ± 1.86	22.45 ± 1.98	23.57 ± 1.84 <sup>e,f</sup>	0.02*
MCHC (%)	33.79 ± 2.21	33.40 ± 2.11	33.11 ± 2.28	0.25
MCV (fl)	63.20 ± 4.70	63.21 ± 4.32	64.23 ± 4.66 <sup>e,f</sup>	0.00*

Data is expressed as mean ± SD; \* Significant (P ≤ 0.05)  
d=Morning (9:00-10:30 am) compared to Afternoon (1:00-2:30 pm)  
e=Morning (9:00-10:30 am) compared to Evening (5:00-6:00 pm)  
f=Afternoon (1:00-2:30 pm) compared to Evening (5:00-6:00 pm)

**Table 1(c):** Comparison of ESR, HB, PCV, WBC, RBC, platelet and RBC indices (MCH, MCHC, MCV) of the female subject at different time of sample collection

attributed to some of the physiological factors in RBC morphology, hormone, posture and diurnal variations, which have been reported to fluctuate the Red Blood Cells, count [11]. Since the amount of fibrinogen in the blood and plasma immunoglobulin directly correlates with the ESR [12], one should consider the fact that amount of these factors in certain time of the day can resist sedimentation by causing the negative charge of the erythrocytes (zeta potential) [13]. In the evening, the RBC tends to decrease due to low blood pressure, stress, age, temperature, and the time of the day (e.g. evening) [14] (Table 1c), thereby leading to slight change in the MCH and MCV. WBC count had slight significant difference in their values. This observation was seen among the males only, which might be due acute psychological stress which activated the endocrine response more profoundly in male subjects than in female subjects [15].

## Conclusion

This study shows that time of sample collection had effect on some of the haematological parameters. These include Erythrocyte Sedimentation Rate (ESR) and white Blood Cell (WBC), which tend to increase in the afternoon hour but are stable only in the morning and evening hours. The Red Blood Cell (RBC) was stable throughout the morning and early afternoon, declining only modestly thereafter. Others parameters like haemoglobin, heamatocrit, platelets, were stable therefore their values were not affected by the time when the samples were collected.

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