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Evaluation of Vitamin B_{12}, Folate, Haematological Parameters and Some Reproductive Hormones in Subjects Attending Fertility Centres in Port Harcourt

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ABSTRACT

Background: **Vitamin B_{12} has proven to have effect in fertility because it improves mature oocyte counts and embryo quality in women and helps in sperm quality in men, so it is efficient to carry out vitamin B_{12} analysis in addition to other hormone profile during routine infertility examination.**

Aim**: The aim of this study was to evaluate vitamin B_{12}, folate, some haematological parameters and some reproductive hormones in men and women attending fertility centres in Port Harcourt.**

Study Design**: This study is a case-control and comparative study, and a random convenient sampling method was employed. A total of two hundred (200) apparently healthy participants within the reproductive age group of 18-44 years attending the fertility clinics of Rivers State University Teaching Hospital (RSUTH) Port Harcourt formerly Braithwaite Memorial Hospital (BMH) which is the only State Government owned teaching hospital in Rivers State located at Forces Avenue Port Harcourt and Save a Life Mission Hospital Port Harcourt a private owned fertility hospital located at Stadium Road, Port Harcourt were recruited for this study.**

Results**: The result showed that there was no statistically significant difference in haematological parameters in cases of infertility in the female and male test groups as compared to control groups ($p>0.05$). The mean ±SD showed non-significant difference of serum folate in the male and female test and control groups of the study ($p>0.05$). This study however observed Vitamin B_{12} level to be statistically significantly lower in infertile females when compared with the controls ($p=0.0078$). There was also a statistically significant difference between the mean values of Vitamin B_{12} in the male test and control groups of this study ($p<0.0001$). Prolactin levels in the females showed a significant difference between the test and control group at ($p<0.0001$), with the mean (± SD) value higher in the test than the control which shows that the infertile female group were mostly having high prolactin levels. Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) were significantly lower in infertile female group as compared with the control ($p<0.0001$). In the study carried out on the principal reproductive male hormone testosterone there was a statistically significant difference between the test and control groups ($p<0.0001$).**

Conclusion**: There was a significant fall in vitamin B_{12} alongside predominant fertility hormones like testosterone in the infertile male subjects. There was also significant reduction in the serum concentration of vitamin B_{12} with a corresponding fall in serum concentration of fertility hormones like; luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the infertile females and a significant rise in the concentration of the female prolactin level. Therefore, Vitamin B_{12} should be included in the evaluation of infertility either primary or secondary alongside other vital conventional parameters usually considered in infertility cases.**

Keywords: Fertility, Folate, Follicle Stimulating Hormone, Infertility, Luteinizing Hormone, Prolactin, Vitamin B_{12}.

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I. Introduction

Infertility can be defined as the inability to conceive after 12 months of unprotected sexual intercourse, it is a common reproductive disorder affecting 15% of couples who attempt to become pregnant [1]. Infertility is described as primary if the couple has never conceived and secondary if they fail to conceive after a previous conception. The prevalence of infertility varies from one community to another and the estimate ranges from 10-15% [2]. In Nigeria, secondary infertility is more prevalent and anovulation accounts for 20% of secondary female infertility [2]. Assisted reproductive technologies (ARTs), which include in-vitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI), have become the main treatment modalities for couples facing infertility in Nigeria [1]. This can actually be avoided if proper laboratory diagnosis has taken place.

Vitamins are the organic compounds required by the human body which are considered as vital nutrients needed in specific amounts. They cannot be synthesized in sufficient amount by the human body, therefore, must be obtained from diet [3]. Thirteen different types of vitamins are known and are classified by their biological and chemical activity; each one of them has a specific role in our body [3]. Vitamins are classified as either water-soluble or fat-soluble. Out of the 13 vitamins, 4 are fat soluble (A, D, E, and K) and the other 9 are water soluble (8 B vitamins and vitamin C). The water-soluble vitamins easily dissolve in water and are excreted from the body rapidly since they are not stored for a long time, except for vitamin B_{12} [4]. On the other hand, fat-soluble vitamins are absorbed in the intestine in the presence of lipid and they are more likely to be stored in the body. As they are stored for a long time, they can lead to hypervitaminosis more than the water-soluble vitamins; some vitamins are vital for the body cell growth and development (e.g. folate and B_{12}) [4].

Vitamin B_{12} (α-(5, 6-dimethylbenzimidazolyl) cobamidcyanide), also named Cyanocobalamin is one of eight known B vitamins [4]. These vitamins are water-soluble and are essential for normal human growth, development, and metabolism [4]. Vitamins are essential vital supplements for fertility and proper healthy life style of individuals [4]. Vitamin B_{12} is synthesized by bacteria or archaea as they contain the required enzymes to assemble this molecular complex. Animal products such as meat and fish are proven food sources of vitamin B_{12} [5]. Vitamin B_{12} is involved in the metabolism of almost all cells in the human body as it is required for DNA synthesis, as well as amino acid and fatty acid metabolism [6]. Therefore, a wide symptomatic spectrum is related to vitamin B_{12} deficiency ranging from fatigue and depression to severe anaemia and memory loss [6]. A more prolonged vitamin B_{12} and serum folate deficiency results in infertility by causing changes in ovulation, development of the ovum and changes leading to defective implantation [4].

Vitamin B_{12} is necessary in both men and women having fertility issues, deficiency can cause infertility, recurrent spontaneous abortion, megaloblastic anaemia (macrocytic anaemia with other features due to impaired cell division) [3]. Vitamin B_{12} enables the release of energy by helping the human body absorb folic acid [7]. Starting pregnancy with an inadequate vitamin B_{12} status may increase risk of birth defects such as neural tube defects (NTD), and may contribute to preterm delivery, although this needs further evaluation [4]. Furthermore, inadequate vitamin B_{12} status in the mother may lead to deficiency in the infant if sufficient fetal stores of vitamin B_{12} are not laid down during pregnancy or are not available in breastmilk [4]. The metabolism of every cell in the body depends on vitamin B_{12}, as it plays a part in the synthesis of fatty acids and energy production [7].

Vitamin B_{12} deficiency is associated with gastric achlorhydria (absence or reduced hydrochloric acid in the gastric secretions), resulting in decreased availability of intrinsic factor [8]. Vitamin B_{12} is considered as a coenzyme for methionine synthase enzyme [8]. This enzyme is required to synthesize methionine from homocysteine to complete the *S*-adenosylmethionine (SAM) cycle. In this cycle, the critical step is the conversion of SAM to *S*-adenosylhomocysteine, which results in the methylation of the main functional macromolecules/molecules in the human body such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), neurotransmitters, lipids, proteins, and amino acids [8].

Folate is the synthetic form of folic acid (Vitamin B_9), it is necessary for fertility in both men and women [1]. It contributes to spermatogenesis [9]. Therefore, receiving sufficient amounts through diet is necessary to avoid low fertility rate. Also, polymorphisms in genes of enzymes involved in folate metabolism could be one reason for fertility complications in some women with unexplained infertility [1].

Folate is necessary for the synthesis of deoxyribonucleic acid (DNA), transfer-ribonucleic acid (tRNA), and the amino acids cysteine and methionine [10]. Thus, it plays an important role in human reproduction. Our body needs folic acid for the synthesis, repair, and methylation of DNA. Moreover, it acts as a cofactor in many vital biological reactions. Folate has an important role in cell division and it is especially needed during infancy and pregnancy [10]. Human body requires folate in order to produce healthy red blood cells and prevent anemia, The importance of folate in reproduction can be appreciated by considering that the existence of the vitamin was first suspected from efforts to explain a potentially fatal megaloblastic anaemia in young pregnant women in India [11]. Today, low maternal folate status during pregnancy and lactation remains a significant cause of maternal morbidity in some communities [11].

The folate status of the neonate tends to be protected at the expense of maternal stores. Nevertheless, there is mounting evidence that inadequate maternal folate status during pregnancy may lead to low infant birth weight, thereby conferring risk of developmental and long-term adverse health outcomes [12]. Moreover, folate-related anaemia during childhood and adolescence might predispose children to further infections and disease [10]. The role of folic acid in prevention of neural tube defects (NTD) is now established, and several studies suggest that this protection may extend to some other birth defects [10].

II. Materials and Methods

A. *Study Design*

This study is a case-control and comparative study and a random convenient sampling method was employed.

B. Study Area

This research was carried out in Port Harcourt, the capital of Rivers State. South-South Nigeria.The samples were collected in Port Harcourt metropolis. Port Harcourt is the largest city in Rivers State, it lies along the Bonny River and is located in the Niger Delta. Port Hacourt has a population of 3,020,000 [13].

C. Study Population

This study was carried out among adults in Port Harcourt attending the fertility clinics of Rivers State University Teaching Hospital (RSUTH), Port Harcourt and Save a Life Mission Hospital, Port Harcourt, a private owned fertility hospital located at Stadium Road, Port Harcourt. Sample size was obtained using a convenient sampling technique. A total of two hundred (200) apparently healthy participants within the reproductive age group of 18 - 44 years comprising of 100 fertile (50 males and 50 females) and 100 infertile (50 males and 50 females) were recruited for this study. A questionnaire was used to get the demographic features of the participants. The infertile group all had medical history of inability to conceive after a period of one year in spite of regular unprotected sexual intercourse and was grouped into; primary and secondary infertility, the fertile groups were all Individuals who just gave birth within a year without a history of delay in conception.

D. Collection of Blood Samples, Storage and Transportation

Blood samples were collected for sampling from women with regular menstrual cycles on the second or third days of their menstrual period (follicular phase) and random sample collected from men. Venous blood specimens were collected from the median cubital vein from all participants. Four milliliters (4 ml) of venous blood were collected from each patient using vacutainer technique by standard method of phlebotomy. Two milliliters (2 ml) were introduced into a plain tube while the remaining 2 ml was introduced into a dipotassium-ethylene diamine tetra-acetic acid (K_2-EDTA) tube and used to assess the full blood count of the subjects within 24 hours of collection. The clotted samples in the plain tubes were centrifuged at 3,000 g for 10 minutes and the serum was harvested into well labeled sterile clean plain tubes.

Samples in plain tubes were preserved in the freezer at -20 ^{o}C temperature. This helped to keep the protein matrix of the sample for a longer period until analysis took place. The serum prolactin, Follicle stimulating hormone (FSH), Luteinizing hormone, testosterone, Vitamin B_{12} and folate were analysed after one month with the reagent kits using Enzyme-Linked Immunosorbent Assay (ELISA) technique.

E. Methodology

1. Determination of Full Blood Count (FBC) using Laborex HM-500×Automated Analyser

Procedure: Sample for analysis was mixed using a vortex mixer and the lid of the sample container was opened and the sample fed into the Auto-analyzer via the probe, the analysis was done by the machine and the results of the analysis displayed at the read-out screen which can also be printed.

2. Determination of Prolactin Concentration Using Prolactin ELISA Kit, Bioassay Technology Laboratory Shangai Korian Biotech Co, Ltd, Shangai, China

Procedure: All reagents, standards and samples were brought to room temperature before use and the test performed at room temperature. The desired number of coated wells was secured in the holder. 50 ul of standard, specimens, and controls were dispensed into appropriates wells and 100 ul of Enzyme Conjugate Reagent was added into each well, the mixture was thoroughly mixed for 10 seconds, and the plate covered with a sealer then incubated at room temperature (18-22 °C) for 60 minutes. After incubation the mixture was removed by flicking plate content into sink, with the use of the wash buffer the plate was rinsed and flicked for 5 times.

The wells were stroked sharply onto absorbent paper towels to remove all residual water droplets. 100 ul of tetramethylbenzidine (TMB) substrate was dispensed into each well and gently mixed for 5 seconds then Incubated at room temperature for 20 minutes. The reaction was stopped by adding 100 ul of Stop solution to each well then it changed to yellow colour completely, optical density was read at 450 nm with a microliter well reader.

3. Determination of Follicle-Stimulating Hormone (FSH) Concentration Using FSH ELISA Kit, Bioassay Technology Laboratory ShangaiKorian Biotech Co, Ltd, Shangai, China

Procedure: Reagents, serum reference calibrators and controls were brought to room temperature (20-27 °C). The microplate wells for each serum reference calibrator, control and patient specimen were formatted and assayed in duplicate. With the use of a pipette 0.050 ml [50 ul] of the appropriate serum reference calibrator, control and specimen were introduced into the assigned wells, 0.100 ml [50 ul] of FSH-Enzyme reagents solution was added to all wells, the microplates were swirled gently for 20-30 seconds to mix and covered with a sealer then kept incubating for 60 minutes at room temperature. After incubation, the contents of the microplate were discarded by decantation then the plate was blotted dry with absorbent paper. 350ul of wash buffer was used to wash the microplate for a total of five times. After washing, 0.100 ml [100 ul] of working substrate solution were added to all the wells at the same order to minimize reaction time differences between wells, then incubated at room temperature for fifteen [15] minutes, 0.050 ml [50 ul] of stop solution was added to each well and mixed gently for 15-20 seconds. Absorbance was read in each well within 30 minutes at 450 nm (using a reference wavelength of 620-630 to minimize well imperfections) in a microplate reader.

4. Determination of Luteinizing Hormone (LH) Concentration Using LH ELISA Kit, Bioassay Technology Laboratory ShangaiKorian Biotech co, Ltd, Shangai, China

Procedure: Reagents, serum reference calibrators, controls and samples were brought to room temperature [20-27 °C]. The microplate wells for each serum reference calibrator, control and patient specimen were formatted and assayed in duplicate. With the use of a micro pipette, 0.050 ml [50 ul] of the appropriate serum reference calibrator, control and specimen was introduced into the assigned wells, 0.100ml [50 ul] of LH-Enzyme reagents solution was added to all wells, the microplates were swirled gently for 20-30 seconds to mix and covered with a sealer then kept incubating for 60 minutes at room temperature. After incubation, the contents of the microplate were discarded by decantation then

the plate was blotted dry with absorbent paper. 350 ul of wash buffer was used to wash the microplate for a total of five times. After washing, 0.100 ml [100 ul] of working substrate solution were added to all the wells at the same order to minimize reaction time differences between wells then incubated at room temperature for fifteen minutes, 0.050 ml [50 ul] of stop solution was added to each well and mixed gently for 15-20 seconds. Absorbance was read in each well within 30 minutes at 450 nm (using a reference wavelength of 620-630 to minimize well imperfections) in a microplate reader.

5. Determination of Testosterone Concentration Using Testosterone ELISA Kit, Bioassay Technology Laboratory ShangaiKorian Biotech co, Ltd, Shangai, China

Procedure: The desired number of coated wells in the holder was properly secured. 25 ul of standards, specimen and controls were dispensed into appropriate well, 50 ul of rabbit anti-testosterone reagent was added to each well and thoroughly mixed for 30 seconds, 100 ul of testosterone-HRP conjugate reagent was added into each well, covered with a sealer and incubated for 60 minutes at 37 °C, after incubation microwells were washed for 5 times with washing buffer [1x]. 100 ul of tetramethylbenzidine (TMB) substrate was dispensed into each well and mixed gently for 10 seconds then incubated at room temperature [18-22 °C] for 20 minutes. The reaction was stopped by adding 100ul of stop solution to each well mixed gently for 30 seconds. Absorbance was read at 450 nm with microliter well reader within 15 minutes.

6. Determination of Vitamin B12 Concentration Using Vitamin B12 ELISA Kit CALBIOTECH Company Limited

Procedure: Reagents and specimens were brought to room temperature before use and gently mixed without foaming. The desired number of coated strips was placed into holder. 50 ul of extracted vitamin B_{12} standards, controls and samples were dispensed into appropriate wells and 50 ul of biotinylated intrinsic factor reagent was added into each well, microplate was mixed gently for 20-30 seconds and covered with a sealer then incubated for 45 minutes at room temperature [20-30 °C]. 50 ul of enzyme conjugate was added into all the wells then gently mixed for 20-30 seconds. After mixing it was incubated for 30 minutes, at room temperature [20-25 °C] then wells were washed 3 times with 1X wash buffer, the wells were struck sharply on absorbent paper to remove residual water droplets. 100 ul of tetramethylbenzidine (TMB) substrate was dispensed into each well and incubated for 15 minutes at room temperature preferably in the dark then 50ul of stop solution was added to each well and gently mixed until a uniform colour was obtained. Absorbance was read at 450 nm within 15 minutes after adding the stop solution.

7. Determination of Folate Concentration Using Folate ELISA Kit CALBIOTECH Company Limited

Procedure: Reagents and specimens were brought to room temperature before use and gently mixed without foaming. The desired number of coated strips was placed into holder. 50ul of extracted folate standards, controls and samples were dispensed into appropriate wells and 50 ul of enzyme conjugate added into each well. 50 ul of biotinylation folate binding protein conjugate was also added, the microplate was gently mixed for 20-30 seconds and covered with a sealer and incubated for 60 minutes, at room temperature [20-25 °C]. The contents of the wells were rinsed 3 times with 1X wash buffer, and the wells sharply stroked on absorbent paper to remove residual water droplets. With the use of a multi-channel pipette, 100 ul of tetramethylbenzidine (TMB) substrate was dispensed into each well and incubated for 15 minutes at room temperature preferably in the dark. 50 ul of stop solution was added to each well and gently mixed until a uniform colour was obtained. The absorbance was read in each well at 450 nm within 15 minutes after the stop solution was added.

F. Statistical Analysis

Graph-Pad Prism 8.0.2.263 version statistical package was used to obtain mean and standard deviation of the study groups. Student t-test was used to determine the statistical difference between the fertile and infertile subjects. Results were presented as mean ± standard deviation (M±SD) and in Tables.

III. Results

A. Demographic Details of Study Population

A total of two hundred (200) participants were recruited for this study, hundred (100) females and hundred (100) males, fifty (50) out of the hundred (100) females where infertile (8 secondary and 42 primary infertility) while fifty (50) where fertile used as the control group, the same with the male participants fifty (50) infertile (8 secondary and 42 primary infertility) and fifty (50) fertile group. The reproductive age range that was used for this study was 18 to 44 years both for the male and female groups. Details of the demographic characteristics of the study population are shown in Table I.

TABLE I: Demographic Characteristics of the Study Population

Participants (N=200)	Infertile Group (N=100)	Control Group (N=100)
Number of Females	50	50
Primary infertile	42	
Secondary Infertility	8	
Number of Males	50	50
Primary infertility	42	
Secondary Infertility	8	
Age Range (Years)	18 - 44	18 - 44
Marital Status (Married)	100	100
Employed	54	55
Unemployed	12	19
Self Employed	34	26

B. Comparison of the Studied Parameters in Females

Table II shows the comparison of vitamin B_{12}, folate, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and some haematological parameters of infertile female group and fertile female control groups. From this study there was no statistically significant difference ($p>0.05$) in the mean values of some of the parameters analysed which include, white blood cell (WBC) count, neutrophil, monocyte, lymphocyte, eosinophil, haematocrit (HCT), haemoglobin (Hb) estimation, mean cell volume(MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin(MCH), platelet count, Red blood cell (RBC) count and folate but there was a statistically significant difference $p<0.05$ in the mean values of Vitamin B_{12}, prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

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TABLE II: COMPARISON OF STUDIED PARAMETERS OF TEST FEMALES AND CONTROL FEMALES

Parameters (Units)	Test (M ±SD)	Control (M ± SD)	p-value
WBC (×10^9/L)	6.946 ± 6.7	6.061 ± 1.8	0.3749
Neutrophil (%)	50.51 ± 15.5	53.96 ± 27.4	0.4423
Monocyte (%)	8.286 ± 6.2	7.346 ± 2.3	0.3228
Lymphocyte (%)	39.30 ± 14.0	43.38 ± 34.6	0.4417
Eosinophil (%)	3.228 ± 5.3	2.338 ± 2.5	0.2865
HCT (%)	31.06 ± 35.9	38.18 ± 56.1	0.5335
HB (g/dl)	11.67 ± 1.2	12.08 ± 1.1	0.0804
MCV (fl)	112.7 ± 171.3	80.98 ± 8.4	0.0888
MCHC (g/dl)	347.9 ± 13.9	408.2 ± 426.2	0.3201
MCH (pg)	28.03 ± 2.6	27.60 ± 5.2	0.6083
Platelet (×10^9/L)	220.5 ± 62.3	210.3 ± 49.8	0.3684
RBC (×10^{12}/L)	5.366 ± 7.3	5.581 ± 6.4	0.9700
Folate (ng/ml)	19.53 ± 10.7	17.64 ± 3.3	0.2400
Vit B$_{12}$ (pg/ml)	420.5 ± 212.2	519.9 ± 148.5	0.0078
Prolactin (mIU/mL)	612.3 ± 427.6	271.7 ± 49.82	<0.0001
FSH (mIU/mL)	10.33 ± 2.7	12.52 ± 2.4	<0.0001
LH (mIU/mL)	7.086 ± 2.4	8.844 ± 1.4	<0.0001

C. Comparison of the Studied Parameters in Males

Table III shows the comparison of vitamin B$_{12}$, folate, testosterone and some haematological parameters of infertile male group and fertile male group. From this study there was no statistically significant difference ($p>0.05$) in the mean values of some of the parameters which are, white blood cell (WBC) count, neutrophil, monocyte, lymphocyte, eosinophil, haematocrit (HCT), haemoglobin (Hb) concentration, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), platelet count, red blood cell (RBC) count and folate but there was a statistically significant difference ($p<0.05$) in the mean and standard deviation values of vitamin B$_{12}$ and testosterone. Details of the result are shown in Table III.

TABLE III: COMPARISON OF STUDIED PARAMETERS OF TEST MALES AND CONTROL MALES

Parameters (Units)	Test (M±SD)	Control (M ±SD)	p-value
WBC (×10^9/L)	6.554 ± 2.2	6.292 ± 2.1	0.5494
Neutrophil (%)	52.13 ± 13.8	50.28 ± 11.8	0.4752
Monocyte (%)	8.116 ± 3.3	7.975 ± 3.1	0.8289
Lymphocyte (%)	48.18 ± 68.0	36.48 ± 14.6	0.2380
Eosinophil (%)	1.964 ± 1.7	1.666 ± 1.7	0.3991
HCT (%)	35.55 ± 3.7	43.69 ± 43.7	0.1933
HB (g/dl)	12.03 ± 2.2	12.56 ± 1.9	0.2168
MCV (fl)	80.96 ± 10.3	94.24 ± 89.2	0.2987
MCHC (g/dl)	346.7 ± 14.2	342.6 ± 15.4	0.1727
MCH (pg)	28.54 ± 2.6	41.18 ± 65.7	0.1775
Platelet (×10^9/L)	253.0 ± 274.8	226.5 ± 79.66	0.5146
RBC (×10^{12}/L)	4.053 ± 0.5	4.835 ± 4.6	0.2378
Folate (ng/ml)	16.86 ± 9.9	17.12 ± 2.5	0.8554
Vit B$_{12}$ (pg/ml)	418.6 ± 246.5	628.9 ± 128.6	<0.0001
Testosterone (ng/ml)	2.216 ± 0.3	3.550 ± 0.5	<0.0001

D. Comparison of the Studied Parameters in Males Based on Infertility Status

Table IV shows the comparison of vitamin B$_{12}$, folate, testosterone, white blood cell, Neutrophil, monocyte, lymphocyte, eosinophil, red blood cell, haematocrit, haemoglobin estimation, platelets, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration of infertile male group based on the fertility status (secondary and primary infertility) of the subjects. From this study there was no statistically significant difference ($p>0.05$) in the mean values of all the parameters analysed. Details of the result are shown in Table IV.

E. Comparison of the Studied Parameters in Females Based on Infertility Status

Table V shows the comparison of vitamin B$_{12}$, folate, prolactin, follicle stimulating hormone, luteinizing hormone, white blood cell, neutrophil, monocyte, lymphocyte, eosinophil, red blood cell, haematocrit, haemoglobin estimation, platelets, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration of infertile female group based on the fertility status (primary and secondary infertility) of the subjects. From this study there was no statistically significant difference ($p>0.05$) in the mean values of all the parameters analysed.

TABLE IV: COMPARISON OF THE STUDIED PARAMETERS IN MALES BASED ON INFERTILITY STATUS

Parameters	Secondary Infertility	Primary Infertility	p-value
WBC (×10^9/L)	6.629 ± 2.4	6.161 ± 1.2	0.5965
Neutrophil (%)	51.08 ±12.7	57.66 ± 18.6	0.2223
Monocyte (%)	8.319 ± 3.4	7.050 ± 2.1	0.3275
Lymphocyte (%)	50.95 ± 73.7	33.64 ± 16.3	0.5155
Eosinophil (%)	1.919 ± 1.6	2.200 ± 2.0	0.6813
HCT (%)	35.14 ± 3.5	37.68 ± 4.3	0.0827
HB (g/dl)	12.66 ± 2.0	11.90 ± 3.3	0.8572
MCV (fl)	81.07 ± 10.7	80.43 ± 7.8	0.8738
MCHC (g/dl)	347.5 ± 13.0	342.9 ± 19.7	0.4097
MCH (pg)	28.70 ± 2.4	27.71 ± 3.6	0.3478
Platelet (×10^9/L)	253.4± 299.7	251.0 ± 5.79	0.9825
RBC (×10^{12}/L)	4.048 ± 0.5	4.081 ± 0.4	0.8634
Folate (ng/ml)	15.86 ± 10.0	22.08 ± 8.0	0.1047
Vit B$_{12}$ (pg/ml)	397.7 ± 240.0	528.4 ± 267.4	0.1717
Testosterone (ng/ml)	2.1 ± 0.3	2.4 ± 0.3	0.0827

TABLE V: COMPARISON OF THE STUDIED PARAMETERS IN FEMALES BASED ON INFERTILITY STATUS

Parameters	Secondary Infertility	Primary Infertility	p-value
WBC (×10^9/L)	5.944 ± 1.5	6.359 ± 3.1	0.5760
Neutrophil (%)	51.45 ± 15.96	45.60 ± 12.95	0.3346
Monocyte (%)	8.417 ± 6.7	7.600 ± 2.9	0.7388
Lymphocyte (%)	38.30 ± 14.1	44.50 ± 12.7	0.2556
Eosinophil (%)	2.690 ± 3.7	6.050 ± 10.1	0.1012
HCT (%)	34.77 ± 10.0	34.36 ± 1.9	0.0909
HB (g/dl)	11.57 ± 1.2	12.19 ± 0.7	0.1938
MCV (fl)	113.8 ± 15.9	169.5 ± 263.0	0.4047
MCHC (g/dl)	347.9 ± 14.6	347.8 ± 10.2	0.9740
MCH (pg)	27.93 ± 2.6	28.58 ± 2.7	0.5349
Platelet (×10^9/L)	219.0 ± 61.52	228.3 ± 70.39	0.7039
RBC (×10^{12}/L)	5.8 ± 7.9	4.4 ± 0.54	0.6127
Folate (ng/ml)	18.52 ± 11.1	24.81 ± 6.90	0.1321
Vit B$_{12}$ (pg/ml)	396.4 ± 197.7	851.3 ± 635.8	0.0649
Prolactin (mIU/mL)	575.9 ± 373.1	851.3 ± 635.8	0.0970
FSH (mIU/mL)	10.39 ± 2.4	9.150 ± 4.9	0.2823
LH (mIU/mL)	7.210 ± 2.4	6.050 ± 3.1	0.2402

IV. DISCUSSION

This study observed that there was no statistically significant difference in white blood cell (WBC) count in cases of female infertility in the test as compared to control group. This may be as a result of the fact that the participants recruited for this research were all apparently healthy individuals. This finding agrees with the study of Ibeh *et al.* [14], in which there was no statistically significant difference in white blood cell count between the test and control groups in cases of infertility in women. There was also no statistically significant difference in the infertile male group

as against the control group. The values in both groups were within the normal range of individuals in the locality of the study.

The study also recorded no statistically significant difference in the mean ± SD between the female test and control groups of white cells (leucocyte) differentials which are neutrophil, monocyte, lymphocyte, and eosinophil) which is in line with the work of Ibeh *et al.* [14]. There was no statistically significant difference between the male test and control groups for neutrophil, monocyte, lymphocyte, and eosinophil. This finding agrees with the study of Ibeh *et al.* [14] but is in contrast with the work of Isaac *et al.* [15] who conducted a study on leucocyte differentials and observed that the males had the highest values of neutrophil, monocyte, lymphocyte, and eosinophil than females. In this study, haematocrit (HCT) level and haemoglobin (Hb) estimation had no statistically significant difference in both female and male test and control groups. The haematocrit level of female test recorded was low as against the control. This could be as a result of the fact that the women were menstruating during collection of the samples, because blood level is reduced during this period. Haemoglobin concentration for the female test recorded as against the control value.

There was no statistically significant difference in the mean ± SD value of the female test and control group of the red blood cell indices which are: Mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH); and the male test and control group of Mean cell volume, Mean cell haemoglobin concentration, Mean cell haemoglobin, mean cell haemoglobin and mean cell volume was higher in male controls than there test group, this findings is in agreement with the study by Isaac *et al.* [15]. There was no statistically significant difference in the platelet count in the mean ± SD value of both the infertile female as against the control group and the infertile male as against the control group. This is in agreement with the work of Ibeh *et al.* [14]. The mean ± SD value of red blood cell (RBC) count was not statistically significantly different in the female test and control group and the male test and control group. This study agrees with the studies by Isaac *et al.* [15] and Ibeh *et al.* [14]. The mean ±SD showed non-significant difference of serum folate ($p = 0.2400$) in the female test. This was not in agreement to the work of Audrey *et al.* [16]. Also, for the male test and control groups of the study, there was no significant difference in the mean value of the test as against the control which supports the previous study of Laurel *et al.*, [17]. This study observed the vitamin B_{12} level to be statistically significantly lower in infertile women when compared with the controls; this is consistent with the work of Ibeh *et al.* [14], that observed that vitamin B_{12} level was significantly lower in infertile women when compared with their control groups ($p<0.05$). There was also a statistically significant difference between the mean values of Vitamin B_{12} in the male test and control group; this agrees with a previous study by Laurel *et al.* [17].

There was a statistically significant difference between the prolactin values of the female test. High prolactin levels (hyperprolactinaemia) recorded in this study might be as a result of some clinical conditions like galactorrhoea, anovulation etc, which might also be the reason for the very low vitamin B_{12} level in women. This work is consistent with the work of Ban *et al.* [18]. Follicle-stimulating hormone (FSH) was significantly lower in infertile female group as compared with the control; and lower test value of luteinizing hormone (LH) as against the control ($p<0.05$). This finding is in agreement with the study carried out by Ban *et al.* [18]. In the study carried out on the principal reproductive male hormone testosterone there was a statistically significant decrease in the test subject against the control subjects (where the testosterone level of the infertile male was lower than the control group), this is similar to a study by Oladosu *et al.* [19] where the mean testosterone level was significantly lower among male partners of infertile couples compared to controls. On comparison of haematological parameters in primary and secondary infertility in both male and female group, there were no statistically significant differences in platelet, red blood cell count, haemoglobin concentration, haematocrit level, leucocyte differentials, red cell indices and white blood cell count between the two groups ($p>0.05$). In the female group it was discovered that there was no statistically significant difference between the primary and secondary group of prolactin, FSH, LH, folate, and vitamin B_{12} ($p>0.05$). Therefore, the fertility status of the subjects did not have any influence on the results this might be because in the study population, primary infertile women were more in number than secondary infertile women. This is not in line with the previous work carried out by Ibeh *et al.* [14], where there was a statistically significant difference in the mean ± SD value between the female primary infertile group and the secondary infertile group ($p<0.05$). In the males there was no significant difference in the testosterone, folate, and vitamin B_{12} level between the primary and secondary infertile group ($p>0.05$) this work is in agreement with the work of Oladosu *et al.* [19]. This means that both primary and secondary infertile men and women have the same risk factor when it comes to infertility.

V. Conclusion

The evaluation of some vital parameters in infertility cases from this study revealed that; haematological parameters assayed did not indicate any significant difference in the infertile male and female subjects. The infertile as well as the fertile subjects had similar haematological profile. Therefore, these parameters are not pointers for infertility. There was a significant fall in vitamin B_{12} alongside predominant fertility hormone like testosterone in the infertile male subjects. There was also significant reduction in the serum concentration of vitamin B_{12} with a corresponding fall in serum concentration of fertility hormones like; luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the infertile females and a significant rise in the concentration of the female prolactin level. The result obtained also indicate that there was no difference in the concentration of vitamin B_{12}, folate and other fertility hormones evaluated when primary and secondary infertile cases were considered. Therefore, Vitamin B_{12} should be included in the evaluation of infertility either primary or secondary alongside other vital conventional parameters usually considered in infertility cases.

VI. Disclaimer

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Consent and Ethical Approval

Informed consent was obtained from apparently healthy subjects prior to enrolment upon approval by the Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

Competing Interests

Authors have declared that no competing interests exist.

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