Relationship between Vitamin B_{12} Deficiency and Infertility on Women Attending Obstetrics and Gynecological Clinic at a Tertiary Hospital in South East, Nigeria.

Nancy Chiatogu Ibeh [1] Charles I. Okafor [2], Ozioma Nwadinigbo [1], Patrick O. Manafa [1]

1.Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi
2.Department of Obstetrics and Gynaecology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria.

ABSTRACT

Introduction: Vitamin B_{12} deficiency is emerging as a growing public health problem in obstetrics. A number of studies show vitamin B_{12} deficiency to be a risk factor in female infertility. Conventionally hormonal assay has been used in the investigation of infertility; this is without recourse to vitamin B_{12} assay. This study therefore aims at estimating vitamin B_{12} level in infertile women attending Obstetrics and Gynecology clinic in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi with the view of determining its role or relationship in female fertility in the study subjects.

Method: A total of 165 subjects with different fertility status who were in the child bearing age were recruited into this cross sectional and comparative study through simple random sampling. The enlisted subjects were then grouped into 4 based on their fertility status: group A (35) had primary infertility, group B (22) had secondary infertility, group C (28) had recurrent miscarriages and group D (80) were non pregnant fertile women as control. Fasting blood (5mls) was collected, 2mls was dispensed into EDTA bottles for FBC determination using Procan PE 6800 analyzer and the remaining 3mls dispensed into plain container, the serum was used for batch vitamin B_{12} estimation by ELISA method.

Results: Vitamin B_{12} (pg/ml) level of the infertile subjects was 232.15±241.13 while the control 406.29±214.58. Comparison using t-test, a statistical difference of ($p < 0.05$) was observed. The Vitamin B_{12} for each of the 4 groups based on their fertility status using ANOVA test statistics: group A was 174.33±156.94; group B had 461.84±277.25, while group C and D were 157.15±214.91 and 406.29±214.58 respectively. The Post Hoc intra group comparison of the 4 groups reveals a statistical difference between groups A (1^0 infertility) & B (2^0 infertility), A & D(control), B & C(recurrent abortion) and C &D ($p < 0.05$). No difference was observed for the RBC, PCV, Hb levels between the test and control groups. The case is same for platelet counts, total WBC and its differentials except in neutrophil count.

Conclusion: This study suggests that lower level in vitamin B_{12} may be associated with infertility particularly in primary and recurrent abortion cases in subjects studied.

Key words: Vitamin B_{12} deficiency, female infertility, pregnancy, childlessness

*Corresponding author: Email: nc.ibeh@unizik.edu.ng Cell Phone: **08061167681; ORCID=None**

Author's contributions: This work was carried out and approved in collaboration between all the authors, **NCI** designed the study; **ON** sourced for funding; **NCI, CIO,** and **ON** wrote the protocol; **NCI, ON** and **POM** contributed in literature search; **NCI** and **POM** did statistical analysis; **NCI, CIO, POM** and **ON** contributed in discussions; **NCI** and **ON** drafted the manuscript; **NCI** supervised the study; **NCI** Wrote the final manuscript; **NCI, CIO, ON**, and **POM** proofread the manuscript.

Received: 10/02, 2019**; Accepted:** 12/11, 2019**; Published:** 12/26, 2019**.**

Citation: Ibeh NC, Okafor CI, Nwadinigbo O, Manafa PO. Relationship between Vitamin B12 Deficiency and Infertility on Women Attending Obstetrics and Gynecological Clinic at a Tertiary Hospital in South East, Nigeria**. J Med Lab Sci, 2019; 29 (3): 1-7**

INTRODUCTION

Over the last few decades, female fertility has declined globally to unprecedented low levels (1, 2). Infertility is defined as the inability to conceive after 12 months of unprotected intercourse (3). The cause of the decline appears to be multi-factorial ranging from late marriage to biological factors, delay in childbearing because of further education, career priorities, or financial concerns in civilized climes (1,4), this trend is gradually creeping in our locality particularly in the southern part of the country were individuals have virtually embraced western culture. According to *Sally* (5) some of the known biological causes among others include endometriosis, pelvic inflammatory disease, polycystic ovary disease and hormonal alterations. Unfortunately, in more than 70% of reported cases; none of these common causes could be identified; suggesting involvement of potentially modifiable associated risk factors (6, 7). *La Veachiaet al.* (8) posited that identifying these modifiable factors may have clinical and public health relevance in infertility studies. Interestingly, several studies suggest that deficiency of certain micronutrients such as zinc, vitamin A, B_6, B_{12}, folate, iron, selenium, iron among others could be the modifiable factors (9, 10, 5, 8)

Vitamin B_{12} a water-soluble vitamin also known as cobalamin, is an important substrate for various enzymes that act as a co factor (adenosylcobalamin) in the conversion of methyl malonyl coA to succinyl coA. Cobalamin is synthesized by some human gut microbes in insufficient quantities (11) in the large intestine (Albert et al 1980), these microbes are majorly bacteria and archaea (12) unfortunately, the cobalamin so produced are not bioavailable to human. The reason has been that the receptors for absorbing the vitamin are found in the small intestine not withstanding that greater proportion of the microbiata are resident in the colon (13). Ultimately diet is instead more likely to constitute the primary source of cobalamin in humans, (14). The vitamin by function is essential in most biological processes such as DNA synthesis, repair and cell division (10). Deficiency therefore results in the accumulation of methylmalonyl coA as well as impaired DNA synthesis (15). Chronic deficiencies could result in inconsistent ovulatory cycles and in extreme case, anovulation. Where conception is achieved, it could cause changes to the development of the ovum and affects implantation and or result in miscarriage of the fetus (10). The miscarriage is postulated to be due to defective methylation and high accumulation of homocysteine levels (10). Elevated homocysteine levels has been known to increase the risk of developing blood clots during pregnancy, and in many cases, clots that occur during pregnancy are what leads to miscarriage. Vitamin B_{12} deficiency is also associated with abnormal estrogen level that interferes with implantation of fertilized egg (5). Vitamin B_{12} deficiency in addition, causes megaloblastic (dimorphic) anemia that manifests as raised MCV and reduced hemoglobin (11). *Kaplan and Basford* (1976) had earlier observed morphological and quantitative neutrophil abnormalities in peripheral blood of individuals with the deficiency (16). The bone marrow characteristically shows megloblastic picture in marked deficiency, and typically all the bone marrow cells are affected. *Richard et al* (1950) used the term “maturation arrest” to describe the phenomena (17).

The objective of this study therefore is to determine the serum levels of Vitamin B_{12} among infertile women attending Obstetrics and Gynaecology clinic in Tertiary Hospital in South East Nigeria, with the view of

determining the role of vitamin B_{12} in female fertility in the study subjects.

METHODS

In this cross sectional and comparative study, a total of 165 subjects with different fertility status who were in the child bearing age were recruited by simple random sampling technique. The enlisted subjects were then grouped into 4 based on their fertility status: thus group A comprised of 35 subjects with primary infertility, group B were 22 with secondary infertility, group C were 28 with recurrent miscarriages and group D were 80 non pregnant fertile women as control. A total volume of 5mls of fasting blood was collected from the study subjects, 2mls was dispensed into EDTA bottles for FBC determination using Procan PE 6800 analyzer and the remaining 3mls dispensed into plain container, allowed to clot undisturbed and the expressed serum was used for batch vitamin B_{12} estimation by ELISA method.

Ethical Consideration:
Ethical clearance was obtained from the institutional ethical committee and written inform consent obtained from the subjects.

Statistical Analysis:
SPSS (version 21) was used to analyze the data generated. Student's t test and ANOVA test statistics was used for comparison of two variables and more than two variables respectively and significant value set at $p \leq 0.005$.

RESULTS

Table 1: Comparison of mean ± SD levels of Vitamin B_{12} and RBC indices of the test and control groups. The vitamin B_{12} value of test group was significantly lower compared with control group ($P < 0.05$), while the mean value of Hb, PCV, RBC, MCH, MCV, MCHC test group showed no significant difference compared with the control group($P < 0.05$).

TABLE 1: VITAMIN B_{12} LEVEL AND RBC INDICES OF THE TEST AND CONTROL GROUPS

Parameters	Test group n=85	Control group n=80	t-test	p-value
Vitamin B_{12} (Pg/ml)	232.15±241.13	406.29±214.58	-3.412	0.001*
HB(g/dl)	10.98±0.99	10.78±1.12	0.865	0.390
PCV (%)	34.53±2.92	34.88±3.37	-0.496	0.621
RBC($X10^{12}$/L)	4.29±0.35	4.38±1.22	-0.453	0.652
MCH(pg)	25.75±3.04	25.85±2.40	-0.155	0.877
MCHC(g/dl)	30.90±4.89	30.43±1.51	0.583	0.561
MCV(fl)	83.87±6.86	84.55±7.47	0.294	0.224

*Significant at $p < 0.05$
Key: PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red blood cell, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration.

Table 2: Comparison of vitamin B_{12}, PCV, Hb, RBC and according to fertility status.
There was no difference between the mean value of vitamin B_{12} in group A and C (p=0.822) and then between B and D (p=0.422). However, a significant difference was observed between A and B/D (P<0.005) were A was significantly reduced compared

with B and D. similarly, there was no a significant difference between C in comparison with B/D (P<0.005). No significant difference was seen between the mean values of PCV, Hb, RBC, among the groups (p>0.005)

TABLE 2: COMPARISM OF VITAMIN B_{12}, PCV, HB, AND RBC ACCORDING TO FERTILTY STATUS.

	PCV (%)	Hb(g/dl)	RBC ($x10^{12}$/L)	Vitamin B_{12}(pg/ml)
Primary infertility (A) n = 35	35.24±2.84	11.24±0.97	4.28±0.34	174.33±156.94
Secondary infertility(B) n= 22	34.54±3.24	10.83±1.27	4.25±0.47	461.84±277.25
Recurrent loss(C) n = 28	33.86±2.85	10.82±0.84	4.33±0.30	157.15±214.91
Control(D) n =80	34.88±3.37	10.78±1.12	4.38±1.23	406.29±214.58
f-value	0.570	0.726	0.082	8.974
p-value	0.636	0.539	0.969	<0.001
A vs B	0.577	0.339	0.811	0.003*
A vs C	0.199	0.246	0.735	0.822
A vs D	0.710	0.153	0.717	0.001*
B vs C	0.580	0.972	0.594	0.001*
B vs D	0.773	0.882	0.685	0.482
C vs D	0.279	0.890	0.835	<0.001*

Key *significant at P < 0.05 PVC = Packed cell volume, Hb = Heamoglobin, RBC = Red blood cell

Table 3: Comparison of mean ± SD of total TWBC (x10^9 /L), Differential count and platelet count of the test and control subject

No difference was seen in TWBC, LYM, and Platelets count of test group compared with the control.

TABLE 3: TWBC, DIFFERENTIAL COUNT AND PLATELET COUNT OF THE TEST AND CONTROL SUBJECTS.

Parameters	Test group (n=85)	Control group (n=80)	t-test	p-value
WBC(X 10^9/L)	5.08±1.96	5.46±1.31	-1.004	0.319
Neutrophil (%)	48.87±12.21	54.31±9.50	-2.225	0.029
Lymphocyte (%)	43.03±7.78	39.18±12.63	1.643	0.104
MID (%)	9.21±3.49	12.98±18.94	-1.239	0.219
Platelet (%)	227.11±69.91	208.76±51.85	1.33	0.186

Key: WBC = white blood cell, MID = Comprising of Eosinophil, Basophil and Monocyte.

DISCUSSION

Cases of infertility have been on the increase and appear to be assuming an alarming proportion in the recent times (1, 2). A significant number of female attendances to Gynecology Clinic have been on account of infertility. Recent studies point vitamin B_{12} deficiency as a risk factor in female infertility. Conventionally hormonal assay has been the first line of thought in the investigation of infertility when other biological factors (5) have been ruled out, this is without recourse to vitamin B_{12} assessment. This study therefore aims at determining vitamin B_{12} level in childless women attending Obstetrics and Gynecology clinic in Tertiary Hospital South East Nigeria, with the view of determining its role in female fertility.

This study observed vitamin B_{12} level to be significantly lower in infertile women when compared with the controls. It has been reported earlier that infertility as a result of vitamin B_{12} deficiency may be associated with cessation of ovulation, distortion in cell division in the ovum that eventually get fertilized or a failure of implantation due to changes in the endometrium (10;17) caused by alteration in DNA synthesis. Benneth et al had reported this vitamin as very key in most biological processes such as DNA synthesis, repair and cell division (10). Deficiency therefore affects any metabolic process involving DNA metabolism. Post Hoc on the infertility status reveals a reduction in values of vitamin B_{12} levels for primary and recurrent fetal lose as against the secondary and control group. The import of this is that vitamin B_{12} deficiency may be the likely cause of infertility in these individual with case of primary and recurrent fetal lost, but not in the secondary groups. One could therefore believe that the case of secondary infertility in the study having vitamin B_{12} values within the value for the control could not have been caused by deficiency or reduction in vitamin B_{12} level. This study would want to assume that infertility in this group could be due to other reason other than vitamin B_{12} deficiency. The study observed no changes in the blood counts. This is consistent with the reports of Kwok *et al.*,(15) and Akash *et al.*, (19) who observed no association between low vitamin B_{12} and any of the hematological indices.

CONCLUSION

The study concludes that that low vitamin B_{12} level could be one of the causes primary infertility and recurrent fetal loss. There was no association with secondary infertility. Estimation of vitamin B_{12} levels is suggested to be included in the battery of assays for the investigation of primary infertile in women.

REFERENCES

1. World Fertility Patterns, Population Division, Department of Economic Social Affairs, United Nations 2015.
2. Ella Schaefer and Deborah Nock, The Impact of Preconceptional Multiple-Micronutrient Supplementation on Female Fertility Clinical Medicine Insights: Women's Health, 2019 Volume: 12
3. Thoma ME, McLain AC, Louis JF, King RB, Trumble AC, Sundaram R, Buck Louis GM. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertile Sterile 2013; 99:1324–31
4. Homan, G, Davies, M, Norman, R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatments: a review. Hum Reprod Update. 2007; 13:209–223.
5. Sally P.M. and stuart J.J., Could it be B_{12}. An Epidemis of misdiagnosis. 2nd ed Chicago, L : Quall Linden publishing 2011.
6. Chavarro, JE, Rich-Edwards, JW, Rosner, BA, Willett, WC. Diet and lifestyle in the prevention of ovulatory disorder infertility. Obstet Gynecol. 2007; 110:1050–1058.
7. Books Gaskins, A, Chavarro, J. Diet and fertility: a review. Am J Obstet Gynecol. 2017; 218:379–389.
8. La Vecchia, I, Paffoni, A, Castiglioni, M. Folate, homocysteine and selected vitamins and minerals status in infertile women. Eur J Contracept Reprod Health Care. 2017;22:70–75.
9. El-Nemr, A, Sabatini, L, Wilson, C, Lower, AM, Al-Shawaf, T, Grudzinskas, JG. Vitamin B12 deficiency and IVF. J Obstet Gynaecol. 1998; 18:192–193.
10. Bennett, M. Vitamin B12 deficiency, infertility and recurrent fetal loss. J Reprod Med. 2001; 46:209–212.
11. Patrick H.Degnan Michiko E.Taga Andrew L.Goodman Vitamin B12 as a Modulator of Gut Microbial Ecology. Cell Metaboloism Volume 20, Issue 5, 4 November 2014, Pages 769-778.
12. Roth J.R, Lawrence J.G., Bobik T.A. Cobalamin (coenzyme B12): synthesis and biological significance Annu. Rev. Microbiol., 50 (1996), pp. 137-181
13. Seetharam B. Alpers , D.H Absorption and transport of cobalamin (vitamin B12) Annu. Rev. Nutr., 2 (1982), pp. 343-369.
14. Antony A.C.Vegetarianism and vitamin B-12 (cobalamin) deficiency Am. J. Clin. Nutr., 78 (2003), pp. 3-6
15. Akash, Krishnamurthy L., Krishnaurthy S.P., Hematological parameters versus serum vitamin B12 levels in the Diagnosis of vitamin B12 Deficiency Neurological deficits. International Journal of science and Research 2016, 5: 567- 569
16. Kaplan SS, Basford RE Effect of vitamin B12 and folic acid deficiencies on neutrophil function.Blood.1976 May;47(5):801-5

17. Richard W. Vilter, Daniel Horrigan, John F. Mueller, Thomas Jarrold, Carl F. Vilter, Virginia Hawkins and Arthur Seaman. Studies on the relationships of vitamin b12, folic acid, thymine, uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias Blood 1950 5:695-717

18. Marwan A, Wael A, Imad A. The association of hyper homocysteinemia with recurrent pregnancy loss. 2016 www.researchgate.net/publication.

19. Kwok T, Cheng JW, Lai WK. Independent effect of vitamin B12 deficiency on hematological status in older Chinese vegetarian women. American Journal of Hematology 2012, 70: 186 -190.