# EVALUATION OF SERUM VITAMIN $B_{12}$ LEVELS IN HORMONAL CONTRACEPTIVE USERS IN SOME HOSPITALS IN KANO METROPOLIS

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**ABSTRACT**
**Aim:** The aim of the study was to compare the levels of Vitamin $B_{12}$in hormonal contraceptive users and women not on hormonal contraceptives in Kano.
**Methods:** A total of 90 participants were recruited for the study; 60 were on hormonal contraceptive (HCP) users and served as the study subjects while 30 were apparently healthy non hormonal contraceptive users recruited as control subjects. Serum Vitamin $B_{12}$levels were evaluated for both groups and the mean levels were compared.
**Results:**Among the 60 HCP users that were enrolled in this study, 29 use implants (48.3%), 14 (23.3%) used injectables;11 (18.3%) use oral pills and 6 (10.0%)participants used intrauterine contraceptive devices. Serum vitamin $B_{12}$ levels of the two groups were determined using human $B_{12}$ specific ELISA kit. The mean ± standard deviation of vitamin $B_{12}$ levels in hormonal contraceptive users was 163.33±53.128 pg/ml while that of non-HCP users was 381.33±198.542 pg/ml.
Statistical analysis for $B_{12}$ levels indicated a statistically significant decrease in oral contraceptive users with a p-value of <0.001. The study also found statistically significant negative correlation (r<1) between duration of hormonal contraceptive use and serum vitamin $B_{12}$ levels of HCPs users with r-value of -0.031 and p-value of 0.020.
**Conclusion:** The present study observed a significant association between hormonal contraceptive use and serum vitamin $B_{12}$ level. Significantly lower serum vitamin $B_{12}$ concentrations were observed in HCP users. Therefore, Vitamin $B_{12}$ supplementation or different contraceptive methods should be considered in women with pre-existing $B_{12}$deficiency or restrictive dietary habits as the deficiency may be worsened by hormonal contraceptive use.
**Keywords**: Contraception, Combined oral contraceptives, Vitamin $B_{12,}$ Vitamin B12 deficiency

## INTRODUCTION

Hormonal contraceptives also known as birth control pills or "the pill" are medications used to prevent pregnancy (Jones, 2011). They contain a combination of the hormones, estrogen and progestin, and are often prescribed to women for prevention of pregnancy along with alleviating common symptoms of ovulation and menstruation, such as pain, acne, cramps, and heavy bleeding (Jones, 2011). Hormonal contraception is usually adopted as a manner of preventing pregnancy by women in their fertility phase mainly ranging from 15-50 years (Sharma, 2018).

Vitamin $B_{12}$(cobalamin) is a cobalt-containing vitamin that is synthesized by microorganisms and exists in different chemical forms in foods of animal origin, including milk, cheese and eggs, as well as artificially fortified foods (Rachel and Bernard, 2017). Vitamin $B_{12}$ functions as a cofactor for methionine synthase, accepting a methyl group from 5-methyltetrahydrofolate (THF) in order to regenerate THF. It also functions as a coenzyme for L-methylmalonyl-CoA mutase in the conversion of L-methylmalonyl-CoA to succinyl CoA (Stephanie *et al*., 2011).

Vitamin $B_{12}$ deficiency can be attributed to various factors such as genetic defects (in the genes involved in $B_{12}$ homeostasis) and low dietary intake of $B_{12}$ or impairment of absorption.

Defects in the gastric mucosa, chronic atrophic gastritis, gastrectomy, malabsorption in the ileum, intestinal stasis, drugs, and intestinal parasites can also lead to impaired $B_{12}$ absorption. Other causes include old age and pernicious anaemia, characterized by autoimmune destruction of gastric parietal cells resulting in a lack of intrinsic factor (IF) which facilitates absorption (Nielsen *et al*., 2012).

Low serum concentrations of vitamin $B_{12}$ (cobalamin) have been observed in users of oral contraceptives (OCs), in women during pregnancy, and in men treated with high doses of ethinylestradiol for prostate cancer (Lussana*et al*., 2003).

Although lower serum cobalamin concentrations have been reported to be associated with hormonal contraceptive use, this reduction in serum cobalamin concentrations may not indicate a true cobalamin deficiency, as evidenced by the lack of clinical symptoms associated with a severe cobalamin deficiency, such as megaloblastic anemia and neurological abnormalities (Stephanie *et al*., 2011).

There are few studies on the effect of hormonal contraceptives on vitamin $B_{12}$ metabolism and bioavailability.

Therefore, the goal of this study is to investigate the effects of hormonal contraceptives on vitamin $B_{12}$ concentrations. Also, since pregnancy often occurs after hormonal contraceptive use is discontinued or when usage is intermittent, it is important to know if hormonal contraceptives impairs vitamin $B_{12}$ status in women of reproductive age, which may negatively impact fetal development.

## MATERIALS AND METHODS

### Study Population

A total of 60 females on hormonal contraceptives attending the family planning clinics of Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase specialist Hospital in Kano were recruited as the study group and 30 women who were not on any form of contraception were recruited as the control group.

### Inclusion Criteria

Womenon active hormonal contraceptive use

### Exclusion Criteria

All women using hormonal contraceptives who did not agree to participate

Nutritional anemia

### Data Collection

Socio-demographic data was collected using a structured questionnaire. Detailed information about the study was explained to the subjects and a written and signed consent to participate was obtained before subject's sample and data was collected.

### Ethical Approval

Ethical clearance to conduct the research was obtained from the research ethical committee of Kano State Ministry of Health.

### Laboratory Analysis

About 5ml of venous blood sample was collected from the ante-cubital vein of each study subject using sterile syringe and needle into gel activator tubes.

Immediately after collection, blood sample contained in gel activator was left standing for clot retraction and was centrifuged at 3000 rpm for 5minutes to separate the serum component which was then collected in pre-labeled plain tubes, and stored at 2-8$^{o}$C until it was ready for assay.Serum vitamin $B_{12}$ was assayed using Human $B_{12}$ specific delayed competitive ELISA assay kit (Accu-bind ELISA test system).

### Statistical Analysis

Data obtainedwas analyzed using SPSS (Statistical package for the social science) version 20.0 software package. Results were expressed as mean and standard deviation. The means of the case and control groups were compared using the student t-test. $P< 0.05$ was considered as statistically significant.

## RESULTS

### General Characteristics

Among the 90 participants, 60 (66.7%) were hormonal contraceptive users (Subjects), and had a mean age of 29.05± 5.893 years while 30 (33.3%) were non-hormonal contraceptive users (Controls), and had a mean age of 24.23±7.505 years.

Table 1 shows the distribution of the participants by age.

Table 2 shows the distribution of the subjects based on the type of hormonal contraception they use. Among the 60 HCP users that were enrolled in this study 29 (48.3%) use implant, injectables were used by 14 (23.3%), oral pills by 11 (18.3%) and intrauterine contraceptive devices by 6 participants (10.0%).

Table 3 shows the mean serum vitamin $B_{12}$ level of subjects and controls and its comparison among the two groups. The case subjects had a mean concentration ± standard deviation of serum vitamin $B_{12}$ of 163.33±53.128 pg/ml, and the controls had a mean vitamin $B_{12}$ concentration of 381.33±198.542 pg/ml, with a P-value of <0.001. This means that vitamin $B_{12}$ concentration of the subjects were significantly lower than the control group.

Table 4 shows the relationship between duration of hormonal contraceptive use and vitamin $B_{12}$ levels of HCP users (Subjects). The findings show a statistically significant negative correlation (r<1) between the duration of hormonal contraceptive use and serum vitamin $B_{12}$ levels of HCPs users with a r-value of -0.031 and p-value of 0.020. Those that use HCPs over a period of 5-60 months have a mean $B_{12}$ concentration ± standard deviation of 168.68± 52.844 pg/ml while those with the highest duration of use of about 173-228 months had a mean $B_{12}$ level 100.00± 0.000 pg/ml.

**Table 1:** Distribution of the study participants (Subjects and control) by age group

| Age groups | SUBJECTS | CONTROL |
|---|---|---|
| 20-29yrs | 33 (55.0%) | 27 (90.0%) |
| 30-39yrs | 24 (40.0%) | 1 (3.3%) |
| 40-49yrs | 3 (5.0%) | 1 (3.3%) |
| 50-59yrs | 0 (0%) | 1 (3.3%) |
| TOTAL | 60 (100%) | 30 (100%) |

**KEY: % (percentage), yrs= Years**

**Table 2:** Distribution of the subjects based on type of hormonal contraceptive

| Contraceptive Methods | Number of subjects | Percentage (%) |
|---|---|---|
| Oral | 11 | 18.3 |
| Implant | 29 | 48.3 |
| Injectables | 14 | 23.3 |
| Intra uterine contraceptive devices (IUCDs) | 6 | 10.0 |
| Total | 60 | 100 |

**Table 3:**Mean serum vitamin $B_{12}$ level (pg/ml) of subjects and controls and its comparison among the two groups

| PARAMETER | SUBJECTS | CONTROL | p-VALUE* |
|---|---|---|---|
| VITAMIN $B_{12}$(pg/ml) | 163.33±53.128 | 381.33±198.542 | <0.001 |

KEY: *independent T-test, P-value= Probability value

**Table 4:**Relationship between duration of hormonal contraceptive use and vitamin $B_{12}$ levels (pg/ml) of HCP users (Subjects)

| Duration (Months) | N | Mean serum $B_{12}$ level | r* | p-value |
|---|---|---|---|---|
| 5-60 | 53 | 168.68± 52.844 | **-0.301** | **0.020** |
| 61-116 | 2 | 150.00±70.711 | | |
| 117-172 | 4 | 105.00± 10.000 | | |
| 173-228 | 1 | 100.00± 0.000 | | |

N= number of subjects, r* = coefficient of correlation (Paired t-test), p-value= probability value

## DISCUSSION

This study has shown that hormonal contraceptive usage is associated with a decrease in vitamin $B_{12}$ concentrations compared to non-users.

The mechanism by which serum vitamin $B_{12}$ is reduced in HCP users is not fully understood. It has been suggested that deficiency of vitamin $B_{12}$ binders in serum is the most likely explanation. This would result in falsely low vitamin $B_{12}$ levels that may not show clinical symptoms of vitamin $B_{12}$ deficiency (Berenson and Rahman, 2012). It has also been hypothesized that the female sex hormones in OCs may affect the production of haptocorrin (which protect $B_{12}$ against the acid while it moves through the stomach), since haptocorrin levels decrease during pregnancy as well (Wilson *et al.,* 2011). Wynn (2005) also suggested that oral pills inhibit the production of transcobalamin-1, a glycoprotein synthesized by leukocytes which is concerned with plasma $B_{12}$ transport.

Cobalamin deficiency can be asymptomatic or may present with some common clinical side effects including neurological disorders like nerve cell damage,seizures, tingling or numbness to the fingers and toes, difficulty in walking, memory loss, and disorientation. Various specific symptoms have also been reported to be related to $B_{12}$ deficiency including optic atrophy, reversible dementia, and myelopathy (subacute combined degeneration of the spinal cord) resulting from an interference in myelin synthesis. Megaloblastic anaemia with an elevated mean corpuscular volume (MCV) is another typical sign of vitamin $B_{12}$ deficiency. Therefore, particular attention needs to be paid to the users of hormonal contraception. The exact biological significance of $B_{12}$ deficiency without clinical signs and symptoms, however, remains unclear.

A study by Green *et al.* (2017) supports these findings with vitamin $B_{12}$ levels of HCP users 33% lower than that of the non-users. This finding also agrees with various previous cross-sectional and case-control studies on the effect of HCPs on serum $B_{12}$ levels that have reported significantly lower serum concentrations of vitamin $B_{12}$ in users of hormonal contraceptives compared to non-users (Lussana*et al.,* 2003; Sütterlin*et al.,* 2003; Berenson and Rahman, 2012;Arthur *et al*., 2013 and Prasad *et al.,*2015). This supports the theory that vitamin $B_{12}$ supplementation could be helpful in HCP users although these findings are not easily transferred to other populations. Furthermore, Riedel *et al.* (2005)also reported that lower serum cobalamin concentrations were associated with oral contraceptive use in a study in which dietary intake and vitamins supplementation was controlled. However, our finding is in contrast to that of Steegers-Theunissen*et al.* (2009)who found similar serum vitamin $B_{12}$ levels in 11 OC users and 15 OC non-users.

The findings from this study also shows an inverse relationship between the duration of hormonal contraceptive use and serum vitamin $B_{12}$ levels of HCPs users. As the duration of hormonal contraceptive use increased, vitamin $B_{12}$ level decreased, as evidenced by the negative coefficient of correlation.

## CONCLUSION

The study concluded that mean serum vitamin $B_{12}$ levels were significantly lower in women using hormonal contraceptives compared to that of healthy non-users (controls). It also shows that the duration of hormonal contraceptive use has a negative impact on serum vitamin $B_{12}$ levels of HCP users.

It is recommended that Vitamin $B_{12}$ supplementation or different contraceptive methods should be considered in women with pre-existing $B_{12}$ deficiency who wish to use hormonal contraceptives.

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