Serum Folate Levels in Hormonal Contraceptive Users in Kano

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ABSTRACT

Hormonal contraceptives (HCPs) are the major class of prescription drugs, used by a large proportion of women starting from early adolescence. Folate is a vitamin with significant roles to play in cell division and amino acid metabolism, under its function in various coenzyme-dependent reactions. This study was aimed at evaluating serum folate level in HCP users and compare these levels to non-HCP users. Sixty healthy females of reproductive age on HCP's were recruited as the test group and 30 non-HCP users were recruited as the control group. The serum Folate levels of the subjects and the controls were determined by human Folate specific ELISA Kit. The results showed a significant decrease in mean serum Folate levels of subjects compared to controls with mean and standard deviation values of 2.3747 ± 1.62129ng/ml and 6.4200 ± 3.69803ng/ml respectively (p <0.001). Thus, based on the findings of the current study, HCP use is associated with a lowered serum Folate level. Therefore, it is recommended that women continue periodic Folate supplementation during HCP use.

Keywords:Folate, folate deficiency, contraceptives, estrogen and progestin

INTRODUCTION

Contraception is the use of different devices, drug agents, sexual practices or surgical procedures to avoid conception or impregnation.[1] Contraception can be hormonal or non-hormonal based. Hormonal contraceptives (HCP's) are synthetic biochemical substances that act on the endocrine system and permit sexual union without resultant pregnancy.[2]

Hormonal based contraceptives may be combined oral contraceptives (OC), injectables, transdermal patch, vaginal ring, intrauterine devices (IUD), or implant. Among the hormonal based contraception methods, combined oral contraceptives are the most frequently used and they contain synthetic Estrogen (ethinylestradiol) and synthetic Progesterone.

Folate is a water-soluble vitamin whose coenzymes serve as donors and acceptors of a myriad of one-carbon entities required for enzymatic reactions including those involved in amino acid metabolism (e.g., homocysteineremethylation to methionine), and DNA synthesis and methylation.[3] Naturally occurring Folate is found in abundant quantity in some food sources, including green leafy vegetables, orange juice, and legumes.[(4)] Impaired Folate metabolism can dramatically alter cell division which is especially evident when cells are rapidly dividing during early embryogenesis.

There have been several reports indicating that OC use is associated with impaired status of some nutrients, including Folate and Vitamins B6 and B12, each of which plays vital roles in fetal development. Since pregnancy often occurs shortly after OC use is discontinued or when use is intermittent, it is important to know if OCs impair Vitamin status in women of reproductive age, which may negatively impact fetal development should pregnancy occur.[5]

There is controversy regarding the potential side effects associated with alteration in Folate levels in women using HCP's. Despite the widespread and increasing acceptance and use of HCPs by women especially in the Northwestern region, metabolic complications arising from these HCPs are poorly understood and not well documented in this region. Hence the need for research aimed at a better understanding of these metabolic derangements associated with HCP use.

Specific Objectives

1. To determine the serum Folate levels in healthy non HCP users (control group).

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2. To determine the serum Folate levels in HCP users (subjects) in some selected hospitals in Kano metropolis.
3. To compare the serum Folate levels between HCP and non-HCP users in some selected hospitals in Kano metropolis.
4. To compare serum Folate levels in different HCP users (subjects).

MATERIALS AND METHOD

Study Area

The study was carried out in Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Specialist Hospital, Kano State. Both hospitals located within Kano Metropolis had an average of 25 OC users daily.

Study Population

The study population involved a test group (women using HCP's) and a control group of apparently-healthy, pre-menopausal women who were had no history of HCP usage.

Inclusion Criteria

Only women of reproductive age using various forms of HCP's for at least six months within Kano metropolis were recruited.

Exclusion Criteria

- Recent Folate supplementation
- Folate-affecting medications like methotrexate
- Neoplastic diseases

Ethical Approval

Ethical approval to conduct the research was obtained from Kano state Hospital Management Board, Research Ethics Committee. The participants' (subjects and controls) written consent was sought before the administration of the questionnaires.

Questionnaire Survey

A structured questionnaire was administered to all the study participants to determine the type of HCP use, the duration of use, and sociodemographic data of the participants. This was done for both the test and control groups.

Sample Size Determination

This was calculated using Rao's formula for minimum sample size estimated in health studies.

$$\text{Where, } N = \left(\frac{ts}{me}\right)^2 = \left(\frac{1.96 \times 1.88}{0.475}\right)^2 = 7.5^2 = 60$$

N= minimum sample size (no of participants) = 60

T = confidence interval on the normal distribution curve and is given as 1.96

me = 5% precision × mean used = 0.05×9.50 = 0.475

s= standard deviation of the parameter used = 1.88

The sample size was calculated to be 60 and 30 subjects were taken as control.

Sample Collection, Processing and Survey

Two (2ml) of venous blood sample was collected aseptically from all the participants and dispensed into plain vacutainer containers. The samples were allowed to clot, retracted and then centrifuged at 3000rmp for 5minutes. The serum was transferred into pre-labelled 2ml plain containers and stored at 2-8 until analysis.

Laboratory Analysis

- Serum sample was assayed for Folate, using Human folate specific Sandwich ELISA assay kit.
- Reagents and samples were allowed to stand at room temperature for 30minutes, the content of wash solution was diluted to 1000ml with distilled water and an extraction agent were prepared by making 1/40 (stabilizing agent/ releasing agent) dilute solution.

- All samples, controls and calibrator were extracted by dispensing 0.1ml (100µl) of all samples into individual test tube, pipetting 0.05ml (50µl) of extracting agent to each test tube and dispensing 0.05ml (50µl) of neutralizing buffer following 15 minutes of incubation while shaking after every addition.
- The wells were numbered with sample identification number including the six Folate calibrators (cal A, B, C, D, E and cal F), 0.05ml of extracted Folate calibrators, control and patients were dispensed into the assigned wells and 0.05ml of Folate biotin reagent were added to all the wells. The contents of the microplate were mixed, covered and incubated for 45 minutes at room temperature after which were added 0.05ml of Folate enzyme reagents to all the wells followed by 20 minutes of incubation. The microplate was washed five times using an automatic plate washer, the plate was blotted, and 0.1ml of Folate substrate reagent was added to all the wells followed by 20 minutes of incubation and the addition of 0.05ml of stop solution to each well. The absorbance in each well was read at 450nm.

Statistical Analysis

All collected data and result obtained where analyzed using SPSS version 20 software. Results were expressed as mean and standard deviation (SD). $P < 0.05$ was considered as a statistically significant difference.

RESULTS

A total of 60 HCP users were recruited as the test group and 30 females with no history of HCP use were recruited as the control group.

Table 1 shows the distribution of cases and control by the type of HCP's used.

Table 2 profiles the duration of use across the subject group

Mean Serum Folate Levels

A statistically significant reduction was observed in the mean serum Folate levels of subjects when compared to the control group (2.3747 ± 1.62129 and 6.4200 ± 3.69803 respectively) (Table 2).

Table 3 shows the comparison of serum Folate levels in different subjects using various forms of HCP's.

Table 1: Distribution of subjects based on the types of HCP's used

Contraceptive methods	Number of subjects	Percentage (%)
Oral	11	18.3
Implant	29	48.3
Injectables	14	23.3
Intrauterine devices	6	10.0
Total	60	100

Table 2: Distribution of subjects by the duration of HCP use

Duration of HCP use (months)	Number of subjects	Percentage (%)
5-60	53	88.3
61-116	2	3.3
117-172	4	6.7
173-228	1	1.7
Total	60	100

Table 3: Mean serum Folate levels of subjects and controls ng/ml

Parameters	Subjects (HCP users) Mean ± SD	Controls Mean ± SD	P-value
Folate	2.3747 ± 1.62129	6.4200 ± 3.69803	<0.001

DISCUSSION

HCP's are synthetic biochemical substances that act on the endocrine system and permit sexual union without resultant pregnancy. Folate functions as a coenzyme in the acceptance, oxidation/reduction and transfer of one-carbon units and are particularly important in amino acid metabolism and the synthesis of nucleic acids. There have been several reports indicating that HCP use is associated with impaired status of several nutrients, including Folate and Vitamins B6 each of which plays vital roles in fetal development.

Our study reports a significant decrease in Folate levels in HCP users. This is consistent with a case-control study carried out in Toronto University Canada, on the impact of HCP's on serum Folate levels that reported significantly lower serum Folate levels in HCP users.[6] It is also in accord with the pioneering work of Shojani *et al.* and another study by Sutterlin *et al.*[7,8]

However, the findings in the current study are in contrast with that of Castren and Rossi who found no significant relationship in serum Folate levels between users of OC's and non-users.[9]

There are various explanations for how HCP may deplete Folate level. Stephanie *et al.* postulated that Folate is poorly absorbed in HCP users.[10] ThepolyglutamylFolate form seems to be the most impaired. OC may impair the enzymatic cleavage by the intestinal Folate conjugate enzyme required for polyglutamyl Folate absorption.

CONCLUSION

In this current study, the serum Folate levels of subjects and controls were assessed and the results show a statistically significant decrease in mean serum Folate levels of subjects compared to the controls.

RECOMMENDATION

This study recommends that women on HCP's should consider Folic acid supplementation especially if they desire to conceive in the future.

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