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Full Length Research Paper

Homocysteine levels of infertile females in Enugu, Enugu State, Nigeria

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ABSTRACT: There are increasing indications that nutritional factors play a significant role in reproduction. Of crucial interest is the homocysteine pathway in which several nutrients are involved. Also, the essential role of homocysteine metabolism in fertility cannot be overemphasized. Therefore, the focus of this study is on Homocysteine levels of infertile female subjects in Enugu, Enugu State, Nigeria. Total numbers of 100 patients between the ages of 18 to 45 years were included, with 50 patients in case and 50 patients in control groups. Competitive Enzyme Linked immunosorbent assay (ELISA) was used for the estimation of homocysteine levels in the study subjects. The hormonal parameters analyzed and compared to the homocysteine levels include Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL), Progesterone (P4), and Estradiol (E4). The homocysteine levels ($p \leq 0.05$) of infertile female patients revealed non-significant increased value (19.2±6.55µmol/ml) when compared to the control (14.0±5.31µmol/ml). FSH and homocysteine showed a significant negative association ($r = -0.409$, $p \leq 0.05$) among the test group while no correlation was seen between FSH and homocysteine in the control group ($r = 0.082$, $p \leq 0.05$). There was a positive association between homocysteine and LH in the test group ($r = 0.315$, $p \leq 0.05$) and a weak negative association between homocysteine and LH in the control group ($r = -0.254$. $p \leq 0.05$). Progesterone (P4) did not show correlation with homocysteine among the test group ($r = 0.016$, $p \leq 0.05$) while the control group showed a strong negative association ($r = -0.679$, $p \leq 0.05$).

Keywords: ELISA, homocysteine, hormone, infertility

INTRODUCTION

Infertility implies an apparent failure of a couple to conceive after one year of unprotected regular intercourse. This is based on the observation that 90% of the normal couples achieve conception within a year (Gnoth *et al*., 2005; Cooper *et al*., 2010). Infertility is considered primary where there is zero conception and secondary in circumstances where there is failure in subsequent attempts after achieving a previous one. A case study of industrialized nations has shown that 15 percent of couple will have infertility challenge of either primary or secondary nature at the same point in the course of their productive lives, another half will not achieve the numbers of children desired (Datta *et al*., 2016). The cause of infertility is attributed to various factors including female (50%), male (20-36%), Idiopathic (25-30%) (Evers, 2002), both male and female (10-40%), and unexplained (10-20%) (Aubuchon *et al*., 2012). The polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders affecting approximately 5-10% of women of reproductive age (12-45 years) and is considered to be one of the leading causes of female subfertility (Scarpitta and Sinagra, 2000). However, insulin resistance (IR) and elevated levels of homocysteine (HCY) may be the major risk factors for the occurrence of atherosclerotic cardiovascular disease (CVD) in women with PCOS (Kely *et al*., 2002; Lergo *et al*., 2001). Also, a primary deficiency of natural folate resulting in an increase of the total homocysteine (tHCY) concentration may be detrimental to the quality of the oocyte, subsequent

fertilization, implantation, embryogenesis and fetal outcome (Hague, 2003). Of course, degeneration of oocyte quality may cause increase in abortion rate and lower rate of pregnancy that is tied closely to age-related reduction in female fertility. Consequently, 50% reduction is witnessed in female fertility between the ages of 25 – 35. Furthermore, there is a significant inverse association between the tHCY concentration in follicular fluid and embryo quality as demonstrated in women undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment (Ebisch *et al*., 2006).

Homocysteine is an intermediate substance formed during the breakdown of the amino acid methionine and may undergo re-methylation to methionine or trans-sulfuration to cystathione and cysteine. About 80% of HCY is bound to albumin in the plasma, whereas the remaining 20% exists as free disulphides. Recent research has shown many non-enzymatic factors which may influence homocysteine levels including age, gender, or sex-steroid environment (Rekha *et al*., 2013).

Increment in homocysteine plasma levels may be as a result of increased or deficient catabolism of co-factors and enzymes defects. Smoking is also linked with elevated levels of homocysteine (Rajdl *et al*., 2016). Other factors that affect homocysteine levels are; high alcohol consumption, inadequate nutrition, coffee intake, obesity, lack of exercise and stress. Data generated from the Norwegian Holland study indicated that non-smokers coupled with small coffee drinking and folate intake have a median homocysteine level of 3.0 to 4.8µmol/L lower than the general population.

In elderly people there is a higher level of homocysteine concentration which may be a result of lifestyle factor in association with malabsorption that can be triggered by atrophic gastritis, reduced kidney function, lower metabolism and other age-related physiological changes.

Therefore, it can be concluded that homocysteine levels are determined by lifestyle, drugs, diseases, age factor and the genetic composition of an individual (Christine *et al*., 2018; ,Cooper *et al*., 2010).

Increased levels of homocysteine can cause several diseases, especially diseases that involve inflammatory process and autoimmune diseases (Bedaiwy *et al*., 2004). Autoimmune diseases such as rheumatoid, diabetes, arthritis, vitiligo and hypothyroidism are linked to increased levels of homocysteine. Also, homocysteine as a product of the methionine cycle has been known to play an important role in cardiovascular disease, neurological diseases, and embryology, and in some diseases in the field of obstetrics and gynecology (Ramakrishnan *et al*., 2006). Of course, elevated levels of homocysteine can lead to increase the risk of recurrent miscarriage in early pregnancy (Ramakrishnan *et al*., 2006). From the foregoing background, the essential role of homocysteine metabolism in fertility cannot be overemphasized. However, the role of homocysteine in female infertility has not been well studied specifically in Nigeria. Thus, this study was carried out to evaluate the HCY levels of infertile females in Enugu, Enugu State, Nigeria.

THEORETICAL BACKGROUND

Normal homocysteine levels

Organisms usually have a reasonable amount of homocysteine present at all times. However, homocysteine levels in the plasma increases from childhood to adulthood with boys having more concentration than girls. The difference between the concentration in men and women between the ages of 40-42 is usually 2µmol/L with men still having higher values and mean concentration of 11µmol/L while the women have a mean concentration of 9umol/L. This difference in concentration may be due to difference in body mass, hormones and lifestyle that are gender specific. But the difference soon diminishes after menopause in women with their concentration remaining lower than that of men (Ducros *et al*., 2002).

Furthermore, homocysteine levels vary between pregnant and non-pregnant women with lower values seen in pregnant women. This may be due to a larger plasma volume, marked increase in the rate of metabolism and increased glomerular filtration. A reference range for homocysteine 5- 15µmol/L was agreed upon after a survey of apparently healthy subjects with different age and lifestyle. But interpretation should always consider the age, gender, lifestyle and symptoms of the patients. Patients who have a higher risk of having hyperhomocysteinemia due to hypothyroidism, malnutrition, renal failure or a family history of early onset of CVD and patients on medication have been given a cut-off of 10umol/L by the Nutrition commission of the American Heart Association (Brandao *et al*., 2011). The elderly may also find this as a helpful cut-off when memory loss begins to set in but a lower cut off (< 10µmol/L) may be considered for women who have had complication in pregnancy.

Management of homocysteine levels

Change in lifestyle is advocated as the first line of action in the monitoring of hyperhomocysteinemia before considering all other causes as this has accounted for a large reduction in levels of homocysteine over time. Hyperhomocystenemia resulting from genetic factors, renal factor and aging may be controlled by vitamin supplements which vary across individuals from the levels estimated within a given population. Reduction of 25% to 30% of homocysteine was seen when apparently

healthy population had vitamin supplement that included folic acid and vitamin B12 in a defined concentration. Vitamin B depletion is common among the elderly. When this occurs, normal homocysteine levels can be achieved again by administration of just vitamin B12 after a few weeks. But when it's a deficiency of folate and vitamin B12 homocysteine levels may not be restored to normal (Stanger *et al*., 200; Kondapaneni *et al*., 2020).

MATERIALS AND METHODS

Subjects

Fifty female subjects suffering from infertility were recruited for this study. They were registered and attending the outpatient department of Department of Obstetrics & Gynecology, University of Nigeria Teaching Hospital, ItukuOzalla Enugu Enugu State.While corresponding fifty apparently healthy women with one or more successful pregnancies without any abortion served as control.

Inclusion criteria

1) Age 18-45 years old.
2) Female subjects with cases of infertility

Exclusion criteria

Females with pelvic inflammatory disease, uncontrolled chronic disease, tuberculosis, previous arterial and/or venous thrombosis before age 40 and diabetes were excluded.

Interventions

After taking consent and informing the patients about the procedure, all patients were subjected to complete history taking, general examination, abdominal and pelvic examination, ultrasound examination for pelvic pathology, hysterosalpingography to rule out defects of the anatomical patency of the genital tract, venous blood sampling for serum follicular stimulating hormone, luteinizing hormone, progesterone & prolactin. Venous blood sampling from patient's antecubital vein after overnight fasting centrifuged for 5 minutes at 2500 revolution per minute and plasma was stored till testing for serum homocysteine levels was done.

Sample collection

It is important to note that the time of sample collection for estimation of homocysteine is not usually affected by ingestion of small quantity of food before blood collection however, a protein rich meal interferes with the results and should be avoided at least 24 hours before sample collection as a protein rich diet increases homocysteine levels greatly after 3 hours of ingestion with maximum values seen within 6-8 hours of ingestion of a protein rich diet up to 15% to 20% and normal values can only be measured after 20 hours. To avoid false positive results, separation of the blood sample after collection should be done immediately and placed in ice packs (Ducros *et al*., 2002). The subjects went through some questioning to determine at what stage of the monthly circle for sampling (Follicular or Luteal). The sample for Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (PRL) were collected on 2nd to the 4th day, while Progesterone (P4) and Estradiol (E4) were collected on the 21st day of the menstrual cycle. The blood (5.0ml) was collected by venepuncture into plain bottle and later separated into another sterile plain bottle after centrifugation at 3000 revolution per minute for 5 minutes. The hormones were analyzed by enzyme immunoassay method for the quantitative concentration in human serum. All samples for homocysteine were separated into another sterile plain bottle labeled and stored in refrigerator at -200°C until the analysis was carried out.

Method

Competitive-ELISA for quantitative determination of Human HCY concentrations in serum based on Elabscience Biotech Human HCY (Homocysteine) ELISA Kit was used. Here, a pre-coated microtiter plate with HCY was used. There was room for competition between the Human HCY present in the blood sample or standard with the fixed amount present on the solid phase for binding sites to antibody specific to Human HCY. Unbound antibodies were then washed off the plate. Addition of Horseradish perioxidase (HRP) to the microtiter plate was done and then incubated. There was also addition to each well of TMB substrate solution. The reaction between the enzyme and substrate was brought to an end when sulphuric acid solution was added and the change in colour measured using a spectrophotometer at 450 nm. The optical density (OD) was measured as concentration of human HCY present in the sample when it was compared to a standard curve. The reagents used include reference standard, reference standard and sample diluents, Concentrated biotinylated detected Ab, biotinylated detected Ab diluent, concentrated HRP conjugate, HRP conjugate diluent, substrate reagent, stop solution. 50µL of standard and sample was added to each well. Immediately 50µL Biotinylated Detection Ab added to each well and incubated for 45 minutes at 37ºC. Aspirated and washed 3 times. To each well 100µL of HRP Conjugate was added and incubated for 30 minutes at 37ºC. Aspirated and washed 5 times. 90µL Substrate Reagent was added and incubated for 15 minutes at 37ºC. 50µL Stop Solution added and read at 450nm immediately.

Table 1: Homocysteine levels in infertility female patients and the control group expressed in mean ± SD, p ≤ 0.05

	Patients (n=50)	Control (n=50)	p-Value
HCY(µmol/L)	19.2±6.55	14.0±5.31	0.328

Table 2: Relationship between HCY and the female hormone profiles in test and control group.

	Test		Control	
	Pearson r	**p-value**	**Pearson r**	**p-value**
LH (mIU/ml)	0.315	0.061	-0.254	0.118
FSH (mIU/ml)	-0.409	0.013	0.082	0.621
PROL (ng/ml)	-0.208	0.223	0.100	0.544
P4 (ng/ml)	0.016	0.926	-0.676	0.000
E2 (pg/ml)	0.367	0.028	-0.225	0.168

Data analysis

Statistical analysis of difference was estimated using students't' test and correlation between variables was studied using Pearson's correlation coefficient test. However, p ≤ 0.05 was considered as statistically significant. Results reported as mean ± SD presented in tables.

RESULTS

The homocysteine levels (p ≤ 0.05) of female infertility patients revealed non-significant increased value (19.2±6.55µmol/ml) when compared with the control (14.0±5.31µmol/ml) (Table 1). The mean concentration of homocysteine showed increased value (19.2±6.55) among infertility female patients when compared with the control group (14.0±5.31), but not significant p>0.05. Table 2 shows that there was a positive association between homocysteine (HCY) and luteinizing hormone (LH) levels among the test group and a weak negative association between HCY and LH among control group, but both are not significant (P ≤ 0.05). Homocysteine and Follicle Stimulating Hormone (FSH) levels among test group showed significant negative association (r = -0.409, p ≤ 0.05). HCY did not correlate with FSH among the control group (r = 0.082, p>0.05). Prolactin (PROL) and Homocysteine levels of test and control group showed weak positive and negative associations respectively (Table 2) (p > 0.05). Progesterone (P4) did not correlate with Homocysteine among test group (r = 0.016, p > 0.05), while the control group showed a strong negative association (r = -0.676, p > 0.05). Estrogen (E2) showed significant positive association with homocysteine among test group (r=0.367, p > 0.05), while the control group showed a weak negative association (r = -0.225, p > 0.05).

DISCUSSION

The mean concentration of homocysteine (HCY) showed increased value among infertile female patients when compared with the control group, but not significant p ≤ 0.05, this finding is consistence with the work of Osunkalu *et al*. ([212015)] though the present study was on primary female infertility patients only. Also, this study is in contrast with studies conducted among women with unexplained infertility in Italy by DUva *et al*. (2007), and Pakistan by QureshiQureshi *et al*. (2010), which showed statistically significant increase in plasma HCY when compared to healthy controls. Serum homocysteine values were also found to be significantly higher in the infertile females (17.29±9.49 µmol/L) as compared to their fertile controls (10.87±4.27 µmol/L) (Dubey *et al*., 2016). Elevated circulating homocysteine or hyperhomocysteinemia (HHCY) is known to be associated with biotoxicity and has been underlined as an emerging risk factor for several diseases such as arterial and/or venous thrombosis, adverse pregnancy outcome, congenital malformations and vascular dementia but its role in unexplained infertility is yet under evaluation (Martinelli, 2001).

The measured gonadotrophins (FSH and LH) among infertile female patients in this study showed significant negative association and non-significant positive association with serum HCY levels respectively, with no association among control group (Table 2). Also, the ovarian steroid (estradiol) showed significant positive association with homocysteine among test group (r=0.367, p ≤ 0.05), while the control group showed a weak negative association (r= -0.225, p ≤ 0.05). Equally, findings from this study corroborate earlier reports by Christodoulakos *et al*. (2006) in which the plasma HCY levels dropped with increasing estrogen level. However, Osunkalu *et al*. (2015) shows that the ovarian steroids did

not show significant association with serum HCY levels in both infertile and fertile women. If the theory associating estrogen in the pathway of HCY metabolism were applicable, it would therefore be expected that infertile women with lower values of serum estrogen have a proportionately higher plasma HCY level. Hyperhomocysteinemia is a known risk factor for atherosclerosis, cardiovascular diseases, stroke, peripheral venous thrombosis and neurodegenerative disease. In pregnancy, it is postulated to be a risk factor for adverse outcomes like early pregnancy loss, neural tube defects, preeclampsia, abruption placenta and intrauterine growth restriction (Das *et al*., 2017). In a current study the prevalence of MTHFR (methylenetetrahydrofolate reductase). Mutation was demonstrated as one which may be responsible for hyperhomocysteinemia. However, analysis revealed high levels of homocysteine even in subjects with normal variant of MTHFR. Therefore, a positive association between MTHFR gene 677 C>T polymorphism and female infertility could not be substantiated in this analysis (Das *et al*., 2017).

Conclusion

Serum homocysteine values were found to be higher in the infertile females as compared to the controls, but not significant. Higher prevalence of homocystinemia in this cohort suggests the increased prevalence of micronutrient deficiency due to inadequate diet or dietary fats in the population. This study has been conducted in a limited number of subjects, as such; the results cannot be extrapolated to a larger population. A larger study is therefore necessary to confirm and validate the findings in this study and help resolve issues related to hyperhomocysteinemia and infertility.

REFERENCES

Aubuchon M, Burney RO, Schust DJ, Yao MWM (2012). Infertility and assisted reproductive technology, Berek and Novak's Gynecology.15th ed. South Asian edition, SAE : Wolters and Kluwer Health. 2012:1139.

Bedaiwy MA, Falcone T (2004). Laboratory testing for endometriosis. Clinica Chimica Acta. 2004; 340:41-56.

Brandao M, Luis F, Margarida P, Cardoso, F (2011). Serum homocysteine concentrations in Portuguese young adults: Reference interval Acta Medica Portuguesa. 2011;24(2):271-8.

Christine CW, Auinger P, Joslin A, Yelpaala Y, Green R (2018). Parkinson study group-datatop investigators. vitamin b12 and homocysteine levels predict different outcomes in early parkinson's disease movement disorders: official journal of the Movement Disorder Society. 2018;33; 33(5):762–770.

Christodoulakos GE, Lambrinoudaki IV, Rizos DA, Alexandrou A, Kountouris AV, Creatsas GC (2006). Endogenous sex steroids and circulating homocysteine in healthy Greek postmenopausal women. HORMONES-ATHENS. 2006;5(1):35.

Cooper T, Noonan E, Von Eckardstein S, Auger J, Baker HG, Behre HM, Vogelsong KM (2010). World Health Organization reference values for human semen characteristics. Human reproduction update. 2010;16(3):231-245.

Das V, Misra D, Agrawal S, Agrawal A, Pandey A (2017). Hyperhomocysteinemia and MTHFR gene 677 C> T polymorphism: questionable role in female infertility. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2017;4(3):683-689.

Datta J, Palmer MJ, Tanton C, Gibson LJ, Jones KG, Macdowall W, Glasier A (2016). Prevalence of infertility and help seeking among 15 000 women and men Human Reproduction. 2016;31(9): 2108–2118.,

Dubey P, Gupta N, Dwivedi S, Swaroop N, Lal P, Thawani V (2016). Hyperhomocysteinemia: a risk factor in unexplained infertility. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2016;2; 2(2):165-171.

Ducros V, Demuth K, Sauvant MP, Quillard M, Caussé E, Candito M,… Gerhardt M (2002). Methods for homocysteine analysis and biological relevance of the results, Journal of Chromatography. 2002;B,781,(1–2):207-226.

DUva M, Di Micco P, Strina I, Alviggi C, Iannuzzo M, Ranieri A, ... De Placido G (2007). Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from Southern Italy: a preliminary report. Thrombosis Journal. 2007;5(1):10.

Ebisch IM, Peters WH, Thomas CM, Wetzels AM, Peer PG, Steegers-Theunissen RP (2006). Homocysteine, glutathione and related thiols affect fertility parameters in the (sub) fertile couple. Hum Reprod. 2006;21; 21(7):1725-33.

Evers JL(2002). Female subfertility. Lancet. 2002;360:151-9.

Gnoth C, Godehart E, Frank-Hermann P (2005). Definition and prevalence of subfertility and infertility. Hum Reprod. 2005;20:1144-7.

Hague WM (2003). Homocysteine and pregnancy. Best Pract. Res. Clin. Obstet. Gynaecol. 2003;17(3): 459-69.

Kely CJ, Speirs A, Gould GW, Petvie JR, Lyall H, Kelly JM (2002). Altered vascular function in young women with polycystic ovarysyndrome. J Clin Endocrinol Metab. 2002;87:742-746.

Kondapaneni V, Gutlapalli SD, Poudel S, Zeb M, Toulassi IA, Cancarevic I (2020). Significance of homocysteine levels in the management of polycystic ovarian syndrome: a literature review. Cureus, 12(10), e11110.

Lergo RS, Kunselman AR, Dunaif A (2001). Prevalence and predictors of dyslipedemia in women with polycystic ovary syndrome. Am J Med. 2001;111:607-613.

Martinelli I (2001). Risk factors in venous thromboembolism. Thrombosis and haemostasis. 2001;86(1):395-403.

Osunkalu VO, Makwe CC, Akinsola OJ, Adewumi A, Akomolafe OF (2015). Preliminary report on plasma homocysteine and hormonal variations in infertile women in Lagos, Nigeria. Journal of Clinical Sciences. 2015;12(1):14.

Qureshi SB, Ahmad M, Qureshi PMA, Memon A. Qazi RA (2010). Hyperhomocysteinaemia, vascular related pregnancy complications and the response to vitamin supplementation in pregnant women of Pakistan. JPMA. The Journal of the Pakistan Medical Association. 2010;60(9):741.

Rajdl D, Racek J, Trefil L, Stehlik P, Dobra J, Babuska V (2016). Effect of Folic Acid, Betaine, Vitamin B_6, and Vitamin B12 on Homocysteine and Dimethylglycine Levels in Middle-Aged Men Drinking White Wine. Nutrients. 2016;8; 8(1):34.

Ramakrishnan S, Sulochana KN, Laksmi S, Selvi R, Nethralaya S, Angayarkanni N (2006). Biochemistry of homocystein in health and diseases. Indian J Biochem Biophys. 2006;43:275-83.

Rekha S, Patel, M L, Pooja G, Amita D, Pushplata S, Natu SM (2013). Correlation between elevated homocysteine levels and insulin resistance in infertile women with or without polycystic ovary syndrome in North Indian population. International Journal of Medicine and Medical Sciences 2013;5; 5(3):116-123.

Scarpitta AM, Sinagra D (2000). Polycystic ovary syndrome: an endocrineand metabolic disease. Gynecol Endocrinol. 2000;14:392-395.

Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, Dierkes J, Weger, M (2004). Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. Zeitschrift fur Kardiologie. 2004;93; 93(6):439–453.