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Hyperhomocysteinemia in Pakistani women suffering from unexplained subfertility

Seema Bibi[1] M.B.B.S., F.C.P.S., Mohammad Ali Pir[2] M.B.B.S., M.P.H., Roshan Ara Qazi[2] M.B.B.S., F.C.P.S., Misbah Bibi Qureshi[3] M.Phil.

1 Department of Obstetrics and Gynaecology, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan.
2 Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan.
3 University of Sindh, Jamshoro, Pakistan.

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Abstract

Background: Hyperhomocysteinemia (hhcy) has been considered as a risk factor for several obstetrical complications such as early pregnancy loss, pre-eclampsia and IUGR. Recently its association with infertility has been underscored in IVF failures; however limited information is available about the relationship of hhcy and subfertility.

Objective: To find out the association between unexplained subfertility and hhcy in Pakistani women.

Materials and methods: This observational study was conducted in Department of Obstetrics and Gynaecology, Liaquat University Hospital Hyderabad from 1st April 2008 to 31st March 2009. Study group consisted of all those women who were subfertile for more then one year, have body mass index less than 25, regular menstrual cycle, normal pelvic examination findings and no past history of pelvic inflammatory disease. Exclusion criteria was male factor subfertility, endocrine and ovulatory dysfunction and tubal blockage. Evaluation was done by semen analysis, pelvic ultrasound scan, hystero-salpingography and hormonal assays. Fasting serum levels of homocysteine were determined using a fluorescence polarization immunoassay.

Results: In total, 61 subjects were enrolled in the study including 49 subfertile women and 12 healthy women. Among subfertile women, 39 (80%) were suffering from primary subfertility while 10 (20%) were complaining of secondary subfertility. Majority of the subjects were young, house wives and residents of Hyderabad city. Mean serum fasting homocysteine levels were significantly higher in women suffering from unexplained subfertility as compared to controls (12.8$\pm$5.1 versus 9.7$\pm$1.7, p-value= 0.04).

Conclusion: Hyperhomocysteinemia was observed in women suffering from unexplained subfertility. However large scale clinical studies are required to confirm the association.

Key words: *Hyperhomocysteinemia, Female infertility, Subfertility.*

Introduction

Homocysteine (hcy) is a sulphur containing amino acid which is not present in naturally proteins. It is derived from demythylatian of methionine, requiring folate, vitamin B_6 and B_{12} as enzymatic co-factors. Malnutrition or malabsorption of folate and /or vitamin B_6 and B_{12} or inherited enzymatic defects such as methylenetetrahydrofolate reductase (MTHFR) or Cystathionine B-synthetase (CBS) deficiency leads to raised hcy levels (1-3). Hyperhomocysteinemia (hhcy) has been associated with several ageing related atherosclerotic, thrombo-embolic and

Corresponding Author:
Seema Bibi, Department of Obstetrics and Gynaecology, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan.
Email: sobgsbibi@ cpsp.edu.pk

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neurodegenerative disorders (4-6). In the field of Obstetrics and Gynaecology, it has been underlined as risk factor for adverse pregnancy outcome like early pregnancy loss, neural tube defects, pre-eclampsia, abruptio placentae and intrauterine growth restriction (7, 8). Researchers also pointed towards the harmful effects of hhcy on female fertility. Exact role of hhcy in the earlier stages of reproductive physiology and in related diseases including subfertility is not clear. Defective follicular development, impaired chorionic villous vascularization, implantation failure and harsh uterine environment were the proposed reasons (9-12). However, limited data is available and mostly focused on women with IVF failures.

There are reports of folic acid, vitamin B6 and vitamin B_{12} related hhcy in Pakistani population (13). Therefore this pilot study was planned to know the baseline data regarding the association of hhcy with subfertility in Pakistani women.

Materials and methods

This observational study was conducted from 1st April 2008 to 31st March 2009 in the Department of Obstetrics and Gynaecology, Liaquat University (LUH) Hyderabad. It is the tertiary referral hospital, with 1385 beds, catering the need of both urban and rural population of Sindh province within the radius of approximately 250Km. During the study, about 45,000 women attended the out patient department for various obstetrical and gynecological problems, including 1152 subfertile women. The sample size was calculated by taking the frequency of unexplained subfertility as 7% in Pakistani population (14). About 61 participants were enrolled for the study, after informed consent. The cases were 49 subjects suffering from unexplained subfertility and 12 women were taken as controls. Control group consisted of healthy women with one or more successful pregnancies, without any obstetrical or medical problems like hypertension or Diabetes mellitus.

Study group consisted of all those women who were subfertile for more than one year, have Body mass index (BMI) less than 25, regular menstrual cycle, normal pelvic examination findings and no past history of pelvic inflammatory disease. Exclusion Criteria were male factor subfertility, endocrine and ovulatory dysfunction and tubal blockage. It was not possible to omit folic acid and vitamin B users from the study, as infertile women attending to our university hospital were initially managed by primary healthcare physicians who prescribe these drugs routinely for every subfertile women. Evaluation was done by semen analysis, pelvic ultrasound scan, hystero-salpingography and hormonal assays i.e. serum follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone and serum prolactin. Venous blood samples were collected in fasting state and serum levels of homocysteine were determined using a Fluorescence polarization immunoassay on the IMx analyzer (Abbott Laboratory Pakistan Ltd) in the Research and Diagnostic Laboratory of Liaquat University of Medical and Health Sciences, Jamshoro. Serum levels of <12 µmol/ liter were considered normal, according to DACH LIGA homocysteine classification (15).

Statistical analysis

Data was entered and analyzed in SPSS version 16. Student's 't' test was used to compare the mean ± standard deviation. P-value of ≤0.05 was considered statistically significant.

Results

In total, 61 subjects were enrolled for the study, including 49 subfertile women and 12 healthy women. Among the subfertile women, 39 (80%) were suffering from primary subfertility while 10 (20%) were complaining of secondary subfertility. Demographic profile of both the groups is shown in table I.

Majority of the subjects were young, residents of Hyderabad city and house wives. Half of the subjects were literate. Mean fasting serum homocysteine level was significantly higher (p-value = 0.04) in cases (12.822±5.1823) compare with this level in controls (9.735 ± 1.7961).

Table I. Demographic profile (n=61).

Variables	Cases (n=49)	Controls (n=12)	p-value
Age *	26.9 ± 5.59	32.2 ± 8.24	0.01
BMI*	24.188±4.7	25.011±1.9	0.56
Duration of marriage*	6.6 ± 5.29	4.5 ± 1.88	0.19
Residence**			
Rural	12 (24.5%)	02 (16.7%)	0.71
Urban	37 (75.5%)	10 (83.3%)	
Education**			
Literate	25 (51%)	5 (41.7%)	0.74
Illiterate	24 (49%)	7 (58.3%)	
Occupation**			
House wife	48 (98%)	10 (83.3%)	0.09
Working	01 (2%)	02 (16.7%)	

BMI: Body mass index.
* Results are expressed as mean ± standard deviation.
** Results are expressed as frequency and percentage.

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Discussion

The study revealed raised serum fasting homocysteine levels in Pakistani women suffering from unexplained subfertility as compared to healthy subjects. Findings of our study support the preliminary work of D'Uva et al on the involvement of hcy metabolism in female reproduction (16). Their study revealed raised mean hcy levels of 21.05±8.78 µmol/ liter in 20 women with unexplained sterility as compared to controls. Mean Serum hcy levels of 12.822 µmol/ liter observed in 49 subfertile women in our study were comparatively less from the above cited study, which might be due to intake of folic acid by the subfertile women in the last three months. Reduction of serum hcy levels by intake of folate and vitamin B6 were also reported by the Korean study where serum hcy levels in infertile women, negatively correlated with total intake of vitamin B_{12}, vitamin B 6 and folate (17).

Exact impact of hcy metabolism on female reproductive function is not clear. However several mechanisms were suggested to induce cellular dysfunction secondary to hhcy i.e. thrombophilia, reduced cell division, inflammatory cytokine production, altered nitric oxide metabolism, oxidative stress, apoptosis and defective methylation reaction (18). Studies have suggested that exposure of ovum to high hcy concentration may have deleterious effects on fertilization and early embryogenesis. Raised hcy levels in ovarian follicular fluid were associated with poor quality of embryo and may influence pregnancy outcome following natural or invitro-fertilization (9, 10). Thrombosis of early decidual or chorionic vessels during early period of pregnancy might be responsible for implantation failure. Qublan and associates, in their study on the role of acquired / inherited thrombophilia in recurrent IVF implantation failure, found raised hcy levels in 60% of women with C677T MTFHR mutation (11).

In a recent study on apparently healthy Pakistani subjects, deficiencies of folate, vitamin B6 and vitamin B_{12} were found to be 39.7%, 52.8% and 6.8% respectively. Hhcy was found in 57.2% and it was negatively correlated with serum levels of vitamin B_{12}, B_6 and folate (13). Prolonged deficiency may cause changes in ovulation or defective implantation leading to infertility (19). Deficiency of these vitamins may be attributed to poverty, high prevalence of intestinal parasitic infections and low intake of fresh fruits and vegetables (20).

Intervention studies have shown that supplementation with folate, vitamin B_{12} and B_6 can lower hcy concentration (21, 22). The Nurses Health study II, on 18,555 participants found that regular use of multivitamins including B-vitamins and folic acid reduces the risk of ovulatory infertility (23). Overcoming micronutrient deficiency in women of reproductive age would be a good option for reducing the problem of subfertility at low cost and short time in a developing country like Pakistan.

Despite the limitation of small sample size, this study not only gave insight about the role of hcy in female reproduction but also points towards the hidden deficiency of vitamin B_{12}, vitamin B_6 and folate in Pakistani women. On the basis of this pilot study, large scale community based studies can be planned to assess the prevalence and causes of vitamin B_{12}, B_6 and folate deficiency and associated hhcy and its consequences on women of reproductive age group, particularly subfertility.

Mass supplementation by fortification of staple food with B-vitamins may prevent micronutrient deficiency related health problems, as many underdeveloped countries have adopted this strategy (24). There is also a need to create awareness in general population about the advantages of healthy and hygienic food eating habits.

Conclusion

Serum fasting hcy levels were found to be raised in young, apparently healthy women who were suffering from subfertility as compared to controls. There is a need to plan further clinical studies on large scale to understand the association of hhcy with unexplained subfertility, along with hcy lowering effect of vitamin B_6 , vitamin B_{12} and folate. Promotion of regular use of B-vitamin and folate by women of reproductive age will be cost effective strategy for the eradication of micronutrient deficiency related health problems including subfertility.

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