Research Article

Hyperhomocysteinemia: a risk factor in unexplained infertility

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ABSTRACT

Background: To study the role of hyperhomocysteinemia in unexplained infertility and the impact of its correction with vitamin and mineral supplementation.

Methods: Total of 60 patients were included with 30 patients in case and 30 patients in control groups. Cases included patients with history of inability to conceive with frequent regular unprotected intercourse for at least 1 year. Controls included age matched parous females with at least one live birth and no history of abortions. Among the patients of unexplained infertility with hyperhomocysteinemia, homocysteine lowering agents were given and outcome studied in the form of lowering of homocysteine levels and number of conceptions.

Results: The mean age was 28.1 years in study and 29.5 years in the control group .Mean level of serum homocysteine was significantly higher in study group than normal fertile women i.e.20.5μmol/l and 10.9 μmol/l respectively. Among the patients of unexplained infertility, 22(73.3%) were found to have range above the normal healthy levels. In these patients homocysteine lowering agents were given for 6 weeks and lowering of mean homocysteine levels was observed which was 10.4 μmol/l. Six (27.3%) patients conceived spontaneously during the follow up period.

Conclusions: Serum homocysteine levels are inversely correlated with infertility. Homocysteine lowering agents have a favourable impact on the outcome of infertility and their use is suggested in cases of unexplained infertility associated with hyperhomocysteinemia.

Keywords: Hyperhomocysteinemia, Unexplained infertility, Homocysteine lowering agents, Female 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.[1] Infertility is primary if conception has never occurred and secondary if the patient fails to conceive after having achieved a previous conception. The incidence of infertility in any community varies between 10% and 15%.[2]

Unexplained infertility is a term applied to an infertile couple whose standard investigations are normal.

Therefore, the diagnosis of unexplained infertility is one of exclusion.[3] The incidence of unexplained infertility is approximately 15-17% among the infertile couples .[4] The cause of infertility is attributed to female factors in 40-55%, male factors in 20-30%, both male and female in 10-40% and unexplained in 10-20%.[5]

Hyperhomocysteinemia (HHCY) 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.[6-11]
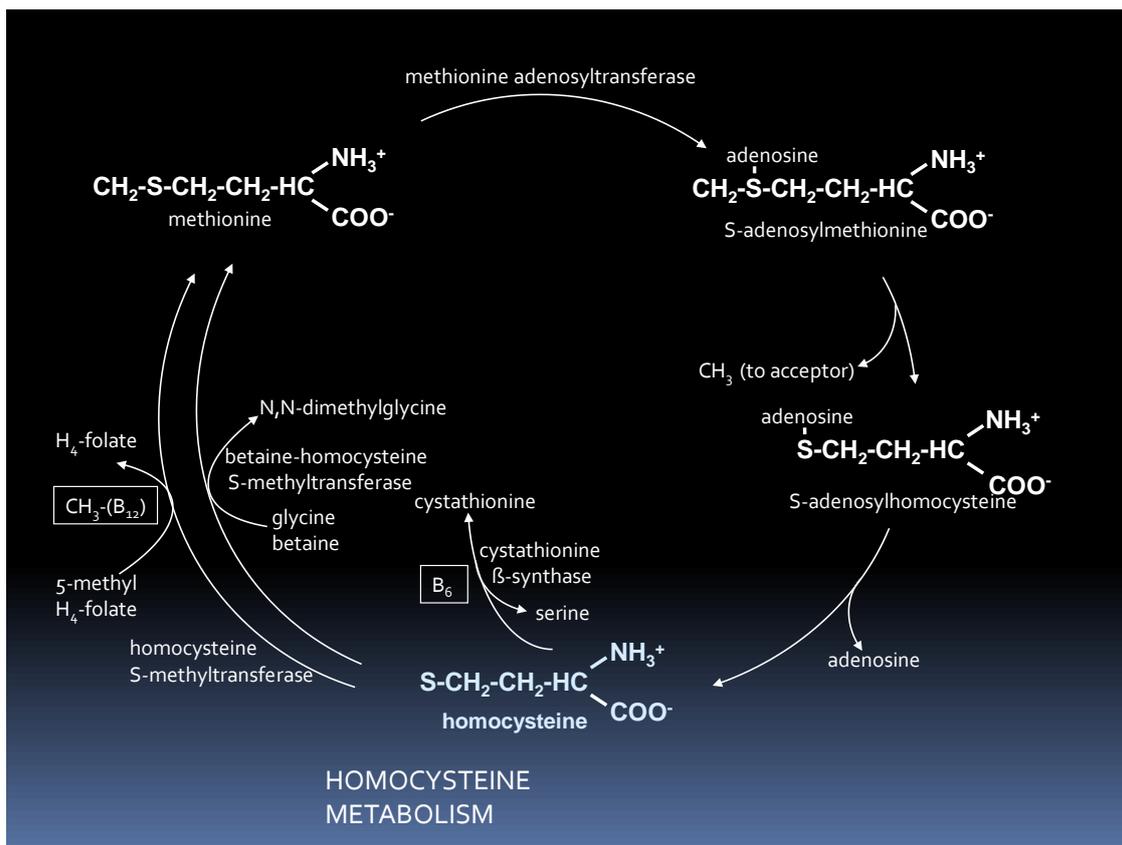
Figure 1: Homocysteine metabolic pathway.

Homocysteine is metabolized through two pathways: remethylation and transsulfuration.[12] Remethylation requires folate and B12 coenzymes; transsulfuration requires pyridoxal-5'-phosphate, the B6 coenzyme. The remethylation pathway requires vitamin B12, folate, and the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). In kidney and liver, homocysteine is also remethylated by the enzyme betaine homocysteine methyltransferase (BHMT), which transfers a methyl group to homocysteine via the demethylation of betaine to dimethylglycine (DMG). The transulfuration pathway requires the enzyme cystathionine-synthase (CBS) and vitamin B6 (pyridoxal-5'- phosphate). Once formed from cystathionine, cysteine can be utilized in protein synthesis and glutathione (GSH) production. Active folate, known as 5-MTHF or 5-methyltetrahydrofolate, works in concert with vitamin B12 as a methyl-group donor in the conversion of homocysteine back to methionine. Normally, about 50% of homocysteine is remethylated; the remaining homocysteine is transsulfurated to cysteine, which requires vitamin B6 as a co-factor. This pathway yields cysteine, which is then used by the body to make glutathione, a powerful antioxidant that protects cellular components against oxidative damage.

Vitamin B2 (riboflavin) and magnesium are also involved in homocysteine metabolism. Thus a person needs different type of vitamins B to keep homocysteine levels low and allow for it to be properly transformed into helpful antioxidants like glutathione. Without adequate levels of vitamin B2, B6, B12, folate and magnesium, dangerous levels of homocysteine may build up in the body. Elevations in intracellular homocysteine concentrations with corresponding increase in blood levels can result from augmented production or reduced metabolism. Elevation in serum levels of homocysteine are typically caused either by genetic defects in the enzymes involved in metabolism or by nutritional deficiencies in vitamin cofactors. It has been observed in literature that these nutritional deficiencies contribute to approximately two thirds of all cases of hyperhomocysteinemia.

Homocysteine has been shown to induce vascular inflammation by enhancing the expression of pro-inflammatory cytokines, such as monocyte chemoattractant protein 1 (MCP-1), which regulates migration and activation of monocytes/macrophages, and interleukin 8 (IL-8), which is an important chemoattractant for neutrophils and T-lymphocyte.[13] Second, homocysteine decreases the bioavailability of nitric oxide (NO), which is one of the major endothelium-dependent vasodilators that is produced by the endothelial isoform of nitric oxide synthase (eNOS).
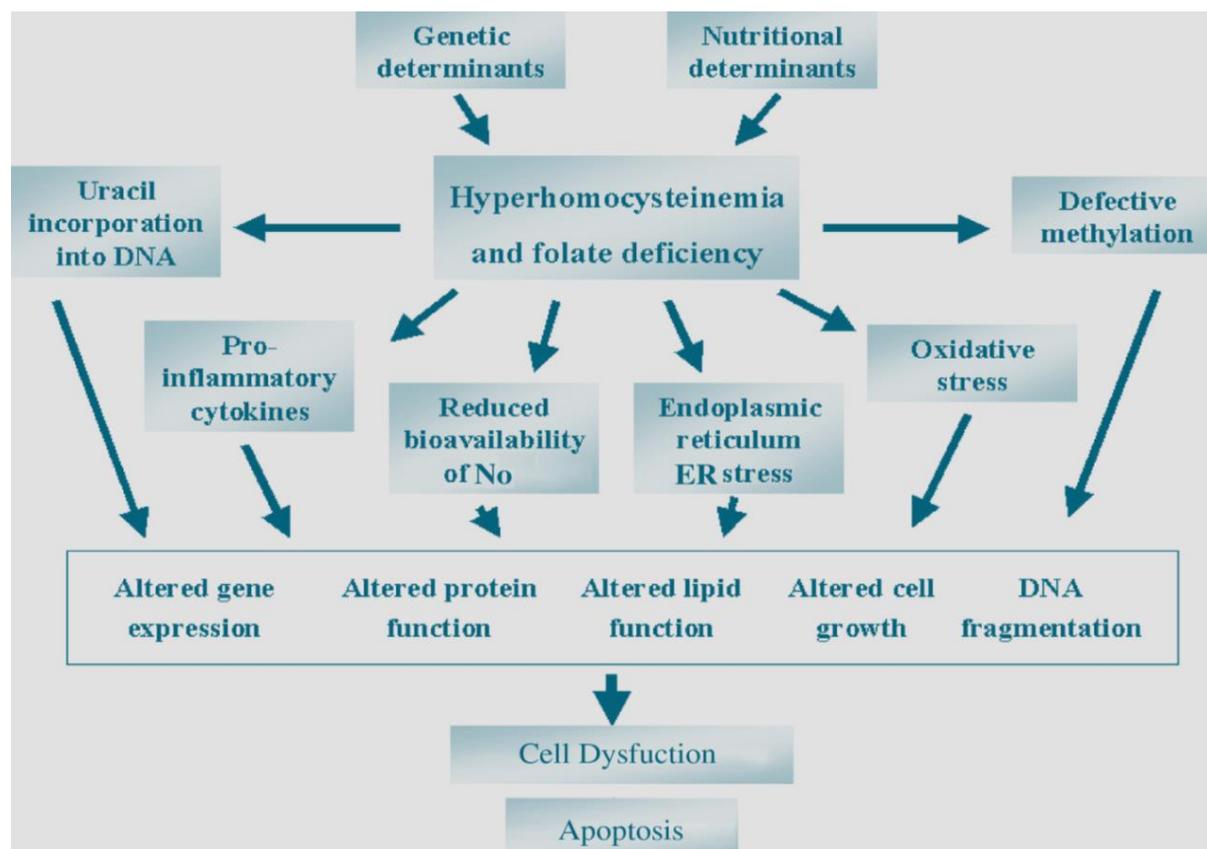
Figure 2: Cellular and molecular mechanisms of hyperhomocysteinemia-induced cell dysfunction.

This effect is mediated by an accelerated oxidative inactivation of NO and/or eNOS or by an increase in serum assymetric dimethylarginine, an endogenous inhibitor of eNOS.[14] Third, hyperhomocysteinemia is found to be associated with the production of reactive oxygen species (ROS) in endothelial and smooth muscle cells. The mechanism of this oxidative stress relies either on auto-oxidation of the highly reactive thiol group of homocysteine or on the formation of intracellular superoxide and peroxyl radicals with concomitant inhibition of cellular antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase.[15,16] Fourth, a more recent concept concerns activation of the unfolded protein response (UPR) that is triggered when unfolded or misfolded proteins accumulate in the endoplasmic reticulum (ER).[17] This ER stress induced after exposure to homocysteine ,further induces the expression of several molecular chaperones and other stress response proteins, which are aimed at restoring correct protein folding or retranslocating defective proteins back to the cytosol for degradation in the proteasomes. In case of a prolonged ER stress, the UPR activates apoptosis by various signaling pathways.[18] Homocysteine-induced endothelial apoptosis probably also involves other mechanisms such as the classical p53 pathway.[19] Furthermore, folate deficiency and genetically determined low MTHFR activity lead to an insufficient remethylation of homocysteine to methionine and a decreased SAM(S-adenosyl methionine) production and

SAM/SAH (S-adenosyl homocysteine) ratio. Insufficient availability of SAM then results in impaired methylation reactions, with multiple consequences. Defective methylation may lead to aberrant gene expression resulting in abnormal fetal development and malignant diseases.[20] Finally, dietary folate deficiency and the resulting decreased cellular synthesis of 5,10-methyleneTHF, as well as reduced MTHFR activity lead to an accumulation of dUMP (deoxy-uridyl monophosphate) and thus to an excessive incorporation of uracil into DNA, with the subsequent repair mechanisms increasing the risk of chromosomal breakage.[21,22]

Many of these mechanisms are involved to cause hyperhomocysteinemia induced infertility. The aim of our study was to assess homocysteine levels in females of unexplained infertility and to study the effect of homocysteine level lowering agents on female infertility.

METHODS

The present study was carried out in unexplained infertile couples attending the outpatient department of Upper India Sugar Exchange Maternity Hospital, Department of Obstetrics & Gynecology, attached to GSVM Medical college, Kanpur between October 2010 and September 2012.

Inclusion criteria:

1) Age 20-40 years old.
2) Cases of Unexplained infertility as diagnosed by ASRM guidelines (2006)[23] for standard infertility evaluation which includes:
a) Absence of male factor: Normal semen analysis as assessed by WHO 2010 guidelines
b) Adequate ovulation using either a mid luteal serum progesterone greater than 10ng/mL, urine testing documenting the LH surge or serial transvaginal ultrasounds to monitor the development and rupture of a dominant ovarian follicle.
c) Normal uterine cavity and patent tubes proved by hysterosalpingography or laparoscopy.
3) Fertile controls included women with one or more successful pregnancies but without gestational complications (e.g. intrauterine growth restriction, preeclampsia, stillbirth and abruptio placentae) and without any abortions.

Exclusion criteria:

Women with associated male factor of infertility, gross pelvic pathology, uncontrolled chronic disease e.g. diabetes mellitus , tuberculosis, history of chronic pelvic inflammatory disease , previous arterial and/or venous thrombosis or history of first degree relatives with arterial and/or venous thrombosis before the age of 65 years were excluded.

Interventions:

After taking consent & 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, TSH, progesterone & prolactin, semen analysis of the patient's husband, venous blood sampling from patient's antecubital vein after overnight fasting centrifuged for 10 min at 2500 rpm and plasma was stored till testing for serum homocysteine levels was done.

In our study we administered folic acid 5mg, vitamin B12 500 microgram, vitamin B6 5 mg to patients with hyperhomocysteinemia (taking 13.5 µmol/l as cut off) and then re-assessed them for homocysteine levels after 6 weeks. The outcomes in terms of conceptions over a period of one year were also noted. The results were analyzed using data software like SPSS and MedCalc.

RESULTS

We compared mean serum homocysteine values among cases and controls and found that mean serum homocysteine levels were 17.27 ± 9.49 µmol/L in the cases and 10.87 µmol/L ± 4.27 µmol/L in the control group. Unpaired t – test was applied. 't' value was 3.37 and degree of freedom was 58 and the result was statistically significant (p value = 0.001 i.e. < 0.05). Mean difference in values observed was 6.4 with 95% confidence interval of the mean difference between 2.60 and 10.20.

Table 1: Comparison of serum homocysteine values among patients of unexplained infertility and fertile patients.

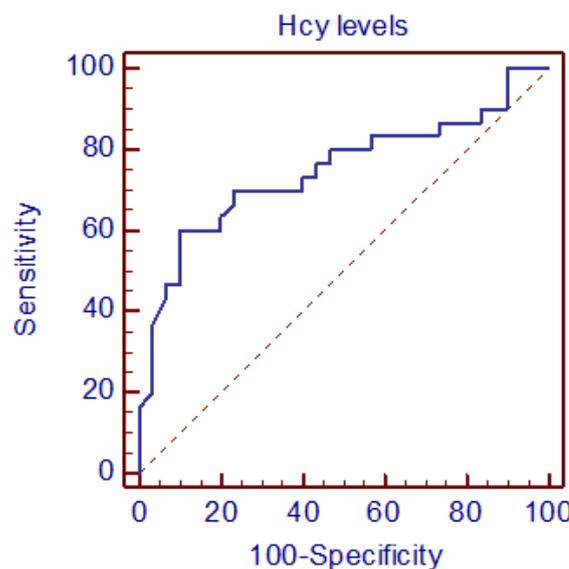| Serum homocysteine (µmol/L)	No. of patients	Mean	Std dev.	t	p
Cases	30	17.27	± 9.49	3.37	0.001
Controls	30	10.87	± 4.27		

Figure 3: ROC curve analysis for serum homocysteine levels and infertility.

ROC curve (Figure 3) was applied to serum homocysteine values in the sample and area under the ROC curve was 0.749 which was significant. z value was 3.774 and significance of p value 0.0002 was observed. Further sensitivity and specificity of the test was calculated at various values and a cut off value of 13.5 was calculated at which maximum sensitivity and specificity was observed (sensitivity 70% and specificity 76.7%).

Majority 22 (73.3%) of patients among the cases had hyperhomocysteinemia while majority 26(86.6%) of controls had normal homocysteine values.

Table 2: Distribution of patients according to homocysteine levels.

Homocysteine levels	No. of cases	%	No. of controls	%
Patients with hyperhomocysteinemia	22	73.33	4	13.33
Patients with normal homocysteine levels	8	26.66	26	86.66
Total	30	100	30	100

These twenty two patients (73.3%) among 30 cases, who were found to have serum homocysteine above the reference range value i.e.>=13.5µmol/l, were given intervention in the form of 6 weeks therapy of homocysteine lowering agents. Paired 't' test was applied to the samples taken before and after and tested for homocysteine levels.

Table 3: Serum homocysteine values before and after treatment intervention.

	Mean (n=22)	Standard Deviation	Paired 't'	*p* value
Before treatment	20.64	± 8.77	7.24	0.0001
After treatment	10.10	± 4.12		

Mean serum homocysteine levels among the 22 cases were 20.64µmol/L ± 8.77 µmol/L before and 10.10 µmol/L ± 4.12 mol/L after the treatment. Mean serum homocysteine lowering was 10.54µmol/L ± 6.83 µmol/L. 95% Confidence Intervals are between 7.51 – 13.5 µmol/L. The result was highly significant (*p* value < 0.001). This result showed that our intervention leads to significant reduction in serum homocysteine values.

Six of the 22 patients conceived in the follow up period of one year after the intervention. However, the patients are still under follow up as the pregnancy outcome of the patients is under study.

Table 4: Number of conceptions among the patients treated for hyperhomocysteinemia.

No of patients with hyperhomocysteinemia treated	Patients conceived	%
22	6	27.27%

DISCUSSION

Homocysteine values have been found to be elevated significantly in patients with unexplained infertility by various statistical analyses in our study i.e. the independent 't' test and ROC curve analysis. Findings of our study were also supported by the preliminary work by D'Uva et al on female reproduction. Their study revealed raised mean homocysteine levels of 21.05±8.78 micromoles /litre in 20 women with unexplained sterility,19.2±6.14µmol/l for patients with recurrent pregnancy loss versus 7.85±3.31µmol/l for controls. Their study gives an indication that infertility and recurrent pregnancy loss are a part of the continuum of the hyperhomocysteinemia induced adverse effects on female reproductive system.[24]

In studies by Bibi et al done in Pakistani women ,mean fasting homocysteine levels observed were significantly higher (p=0.04) in cases (12.82±5.18 micromoles /litre) compared with controls (9.735±1.80 micromoles/litre) which was in concordance with our study.[25]

In our study among the infertile Indian females of unexplained infertility, we found the prevalence of hyperhomocysteinemia to be 73.3%.Refsum H et al in a study of 100 people from Pune , India reported that 77% had plasma homocysteine concentrations >15 micromoles/litre.[26]

In our study intervention in the form of folic acid, vitamin B6, vitamin B12, and zinc lead to significant lowering of serum homocysteine levels. Shidfar F et al in their study concluded that folate supplementation decreases the serum levels of homocysteine. They studied the effect of folate supplementation versus placebo on 40 hyper-cholestrolemic subjects. Their results were similar to that of our study. [27]In studies by Chait A et al supplementation of vitamin B6, B12 & folate rich diet lead to significant reduction in homocysteine levels of studied group.[28]

CONCLUSIONS

Serum homocysteine values were found to be significantly higher in the infertile females (17.29±9.49 micromoles/litre) as compared to their fertile controls (10.87±4.27 micromoles/litre). A critical level of 13.5 µmol/l is suggested as the cut off for intervention by this study.

Higher than usual prevalence of homocystinemia in this cohort suggests the increased prevalence of micronutrient deficiency due to inadequate diet or dietary fads in the population. Micronutrient supplementation in the form of Vitamin B6, B12 and folic acid is helpful in reducing homocysteine values in patients with

hyperhomocysteinemia. However, large scale studies are required to establish the role of hyperhomocysteinemia in unexplained infertility.

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