The Significance Of Maternal Total Serum Homocysteine Level In Iraqi Mothers Who Had Previous Babies With Neural Tube Defects

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Summary:

Background*: Neural tube defects (NTDs) are said to be inherited in a multifactorial fashion, i.e. genetic-environmental interaction. Maternal nutritional deficiencies had long been reported to cause NTDs, especially folate deficiency during early pregnancy. More attention had been paid to the exact mechanism by which this deficiency state causes these defects in the developing embryo. The most significant of all researches was that connecting reduced folate and increased homocysteine level in maternal serum on one hand and the risk of developing a NTD baby on the other hand.*

Objectives : *to determine the significance of homocysteine level in Iraqi mothers who gave birth to babies with NTDs as compared to normal controls.*

Patients, Materials and Methods*: Fifty Iraqi women having babies born with NTDS, referred to the genetic clinic in Baghdad Teaching Hospital, were included in this study (the study group) as well as 37 healthy women having normal children (the control group). This study was conducted from November, 2002 till October, 2003. Analysis of total serum homocysteine level for all women was done using a computerized HPLC system.*

Results *: the age of women in both groups was comparable (mean+SD in the study group was 26.2+5.14 years vs. 26.3+4.57 years in the controls). Among the study group, 4 (8%) had normal tHcy level; 44 (88%) had mildly elevated level, and only 2 (4%) had moderately elevated tHcy level, while all (100%) women in the control group had their tHcy level within normal level. This difference was statistically highly significant ($p<0.001$).*

Conclusions : *Women become at an increased risk of delivering a baby with NTD when having an elevated tHcy level in their sera, and that tHcy level is an important marker in maternal serum that is associated significantly with pregnancy outcome.*

Keywords: *Neural tube defects (NTDs), total serum homocysteine level, maternal serum level*

Introduction:

NTDs account for most congenital anomalies of the CNS and result from failure of the neural tube to close spontaneously between the 3rd and 4th weeks of in utero development [1].

The etiology of NTDs is said to be multifactorial, i.e. it is a genetic-environmental interaction. A polygenic predisposition provides a background risk with a threshold effect which is exceeded by an environmental trigger [2].

Many investigators had pointed to maternal deficiency (especially for folate) as an important aetilogical factor for the development of NTDs [3, 4]; many others tried to pin-point the exact mechanism by which folate deficiency can cause NTDs.

The most striking research had provided an overwhelming evidence for the importance of maternal diet, particularly the use of folic acid during pregnancy [5].

Folate promotes re-methylation of homocysteine (Hcy), a cytotoxic amino acid that can induce DNA strand breakage, oxidative stress and apoptosis [6].

Hyperhomocysteinaemia is also associated with adverse pregnancy outcome, such as spontaneous early abortion, placental vasculopathy and birth defects. It is not only neural tube defects but also cardiac malformations and cleft lip and/or palate, which are associated with higher homocysteine levels than in controls [6].

The recent identification of several, fairly common polymorphisms affecting the genes of enzymes participating in the homocysteine metabolism, and resulting in decreased enzyme activity has also lent the research some impetus. Some of these variations are shown to be associated with increased incidence of the mentioned conditions, such as birth defects [6].

Homocysteine accumulation can be due to dietary (e.g. low folate) or to genetic factors, i.e. it is inherited in a multifactorial pattern [6]. Low folate and elevated Hcy levels are risk factors for NTDs [7]. This risk is modulated by maternal and thus fetal folate levels provided by diet or supplement [8].

The upper limit, i.e. the cut-off value of serum Hcy level is 15 μmol/L [6].

In this study, we aimed to determine the significance of maternal total serum Hcy level in Iraqi women who had previous babies born with NTDs as compared to normal controls.

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Patients, Materials & Methods :

This study was performed in the College of Medicine, Baghdad University. It included 50 Iraqi women who gave birth to a NTD baby. They were referred to the Genetic Clinic, Baghdad Teaching Hospital for genetic counseling during the period from November, 2002 till October, 2003 and were referred to as 'the study group'.
Another group of women, namely the 'control group' consisted of 37 healthy women, whose children were all normal. They were collected from the outpatient clinic at Baghdad Teaching Hospital.
From each woman, a detailed history was taken and a general physical examination was made.
Then, a 3-5 ml of venous blood was withdrawn and rapidly centrifuged with a cooling centrifuge at 3000 RPM for 15 minutes. Serum was then separated and kept in deep freeze (-20^{o}C) till the time of analysis of serum total Hcy level using a computerized high performance liquid chromatography (HPLC) system. This test was done at the Research Center, Iraqi Medical College, Al-Nahrain University.
Statistical analysis was performed on the data using SPSS computer software version 10. P-value of < 0.05 was considered to be statistically significant and a value <0.001 was statistically highly significant.

RESULTS

The age range of women from the study group was from (19-39) years with a mean ± SD of (26.2±5.14); while that of the control group was (19-38) years with a mean ± SD of (26.3±4.57). Both age groups were comparable; Fig. (1).

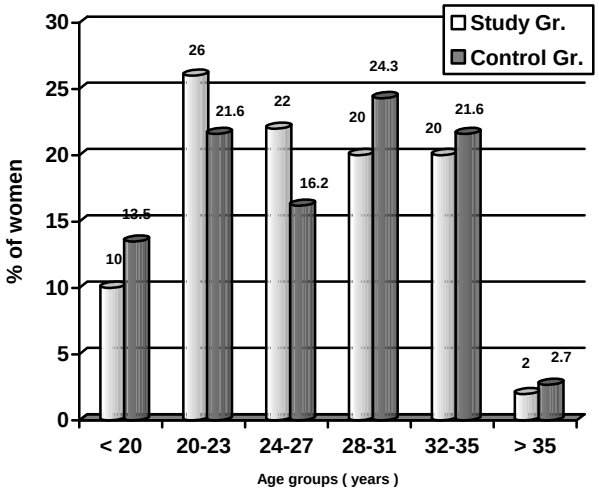

Figure (1) : Distribution of women in both groups according to their age

Examination of total serum homocysteine level in mothers with babies with NTDs showed the following :
The mean ± SD value of homocysteine in the study group was 20.59 ± 4.27, while that of the control group was 10.11 ± 0.74.
Only 4 women of the study group had normal total serum homocysteine level (8%), while the remaining 46 women had abnormal level (i.e. > 15 µmol/l) (92%).
In the control group, all women had normal total homocysteine level in their sera (100%). This difference was statistically highly significant (p<0.001), Figure (2).

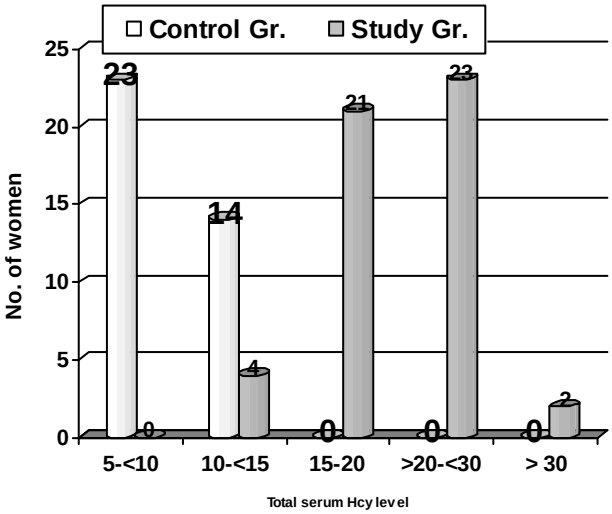

Figure (2) : Distribution of women in both groups according
to their total serum homocysteine level

Hcy level was divided into the following [9] :
15-30 µmol/l = vitamin and nutritional deficiency (mild elevation)
30-100 µmol/l= heterozygous homocysteinuria (moderate elevation)
> 100 µmol/l = homozygous homocysteinuria (severe elevation)

Twenty-one of women in the study group (48%) had total serum Hcy level between 15-20 µmol/l, while 17 (34%) had values between 20-25 µmol/l, 6 (12%) had their serum total Hcy levels between 25-30, and the remaining 2 (4%) had the level > 30 µmol/l (30.23 and 31.33 µmol/l respectively), Fig. (2).
Accordingly, 4 had normal tHcy level, 44 women in our study group had mildly elevated total serum homocysteine level (88%%), and the last two had moderately elevated Hcy level in their sera (4%).

Discussion:
Maternal Age :
The highest number of women in the study group lies between 20-23 years (26%) followed by the age

group 24-27 years (22%). Both constitute 48%. This may suggest that women in their early and mid-twenties are more likely to seek for genetic counseling after delivering an abnormal baby and be more interested in prevention of further abnormal pregnancy outcome whenever possible, because they are at their early family formation, which is in agreement with 2 other Iraqi studies [10, 11]. Other worldwide studies showed an incidence of NTD birth in women around the twenties and at the end of their reproductive life with a U-shaped distribution [12; 13; 14; 15; 16], which could be explained by the fact that in our population, older women do not ask for genetic counseling after having an abnormal baby in contrast to other parts of the world.

Homocysteine Determination :

Association of an elevated Hcy level in women having previous babies with NTDs is significant alongside with a reduced folate level [5], and even the level of Hcy is not a factor of dietary intake only but it follows a multifactorial basis [6].

Four (8%) of women in the study group had normal level, 88% had mildly elevated tHcy level, and only 2 (4%) had moderately elevated level.

This is in agreement with a previous Irish study in that most women with NTD had mildly elevated tHcy level in their sera [17].

Obesity and homoysteine level :

Since tHcy level may increase in obese women and in mothers who delivered a NTD baby [6], and since obese women constituted 34% of the study group and 13.5% among the controls, it is important to know whether the rise in tHcy level is due to obesity itself or to its association with NTDs or to the effect of both factors together.

The mean $\pm$ SD of tHcy level in obese women only among the study group was 23.76 $\pm$ 6.9 µmol/l while that of the control group was 14.766 $\pm$ 0.76 µmol/l, with a statistical significant difference ($p=0.0099$).

Among the rest of the study group (excluding obese women), the mean$\pm$SD tHcy level was 19.46$\pm$3.86 µmol/l while that of the control was 9.26$\pm$1.2 µmol/l, with a statistically highly significant difference ($p<0.001$). This signifies the association between tHcy level in those mothers and the risk for NTD development irrespective of being obese.

Conclusions:

1. Women become at a higher risk for having a baby with NTD when their serum homocysteine level rises.
2. Total Hcy level is a significant marker in maternal serum even when they are not currently pregnant, pointing to a defect in folate metabolism rather than a simple nutritional deficiency, but most cases are preventable by vitamin supplementation even if it is due to an enzyme defect or gene polymorphism.

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