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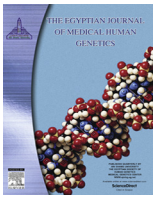

Original article

Abnormal maternal biomarkers of homocysteine and methionine metabolism and the risk of congenital heart defects

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ABSTRACT

Background: Recent advances in genetic technology have had a significant impact on the practice of clinical genetics and the diagnosis of genetic syndromes associated with cardiac malformations.
Aim: The present study was aimed to determine whether biomarkers of the folic acid pathway, including homocysteine and methionine metabolism are altered among non pregnant women who have had a previous pregnancy affected by congenital heart defects.
Subjects and methods: The study was conducted on 50 women attending the Medical Genetics center and the Pediatric Cardiology Clinic, Faculty of Medicine, Ain Shams University for follow up. Mothers were subdivided into: *Group 1 (Cases):* 25 mothers with a history of congenital heart defects in previous children. *Group 2 (Controls):* 25 mothers and their children didn't have any birth defects including congenital heart defects. In both groups women will be excluded: If they were pregnant or taking folate antagonist medications (antiepileptic drugs) or vitamin supplementations at the time of the study. Measurement of plasma concentration of: Vitamin B-12, folic acid, Homocysteine, Methionine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), by using Radio immunoassay kit was done.
Results: There is a significant difference between cases and controls as regards history of early neonatal deaths (28%) in cases versus (4%) in controls ($P < 0.05$). The study also revealed that the most frequent congenital cardiovascular malformation is VSD (32%) followed by ASD (20%).As regards biomarker concentrations all, were significantly different between case and control subjects except for methionine.
Conclusion: An elevated levels of maternal homocysteine is an independent risk factor for congenital heart defects. Finally: There is an increasing need for professionals to apply and interpret genetic testing in a clinically meaningful way for prevention of congenital heart defects.

1. Introduction

Congenital heart defects (CHD) are the most common of all congenital anomalies, representing a major global health problem. Heart morphogenesis is a complex process whose disturbance can produce a range of CHD from harmless to fatal ones [1]. In addition CHD accounts for the majority of deaths from congenital defects in childhood, being six times more common than chromosomal abnormalities and four more common than neural tube defects [2]. Reported birth prevalence of CHD varies widely among studies worldwide [3]. The estimate of 8 per 1000 live births is generally accepted as the best approximation [4]. In Egypt the reported birth prevalence of congenital malformations of the circulatory system is 0.13 per 1000 [5].

The etiologies of the majority of heart defects remain unknown despite their sizable contribution to child morbidity and infant mortality [6]. About 80% of congenital heart disease (CHD) is multifactorial and arises through various combinations of genetic and environmental contributors [7].

Recent advances in genetic technology have had a significant impact on the practice of clinical genetics and the diagnosis of genetic syndromes associated with cardiac malformations as well as sporadic congenital heart disease. These new discoveries have also expanded our understanding of the contribution of genetic

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variation, susceptibility alleles, and epigenetics to isolated congenital heart disease [8]. Scientists are making progress in understanding the genetics of heart defects. Since the 1990s, they have identified about 10 gene mutations that can cause isolated congenital heart disease [9]. In addition environmental factors can contribute to congenital heart defects. Women who contract rubella during the first three months of pregnancy have a high risk of having a baby with a heart defect. Other viral infections, such as the flu, also may contribute. Exposure to certain industrial chemicals (solvents), may play a role. Some studies suggest that drinking alcohol or using cocaine in pregnancy increase the risk of heart defects [10]. Although genetic and environmental factors are involved in the etiology of CHD, only approximately 15% can be attributed to a known cause [7].

Previous epidemiological studies showed that periconceptional use of multivitamins containing folic acid reduces the risk of having a child with CHD [11,12]. Shaw et al., [13] were the first to show that the maternal use of these vitamins during the sensitive period of heart development reduced the risk of conotruncal heart defects in particular. Hernandez-Diaz et al., [14] also suggested that folate is a key factor in cardiovascular development by showing an increased risk of CHD after maternal exposure to folate antagonists during the first trimester of pregnancy. The existing evidence for an association between folic acid and congenital heart defects, however, is still inconclusive [10].

Homocysteine is the product of the intracellular methionine cycle in which methionine is initially activated by ATP to S-adenosylmethionine (SAM), the primary methyl donor for essential methyl transferase reactions. After methyl transfer, SAM is converted to S-adenosylhomocyteine (SAH). The sole source of homocysteine in the body is the hydrolysis of SAH. Interestingly, the equilibrium dynamics favor the reverse reaction, thus elevated homocysteine concentrations cause SAH to accumulate. Increased SAH is a potent product inhibitor of cellular methyl transferases which during organogenesis can alter gene expression, cell differentiation and apoptosis which are associated with congenital heart defects [15].

The aim of the current study is to determine whether biomarkers of the folic acid pathway, including homocysteine and methionine metabolism are altered among non pregnant women who have had a previous pregnancy affected by congenital heart defects or not. The results will be compared with those of women without such a history. This may shed light on new insights into mechanisms that confer an increased risk of having pregnancies affected by congenital heart defects.

2. Subjects and methods

The work has been carried out on 50 mothers. They are divided into two groups who were attending or were following up in the Medical Genetics center and the Pediatric Cardiology Clinic, Faculty of Medicine, Ain Shams University, to establish a maternal risk profile for nonsyndromic congenital heart defects that would enhance current preventive strategies. Their age ranged from 20 to 39 years with the mean of 26.59± years. Mothers were divided into:

Group 1 *(Cases):* 25 mothers with a history of congenital heart defects in previous children.

Group 2 *(Controls):* 25 mothers whose previous children were unaffected by any birth defect including congenital heart defects.

For both groups Time of last pregnancy before participating in the study ranges between 11 and 18 month.

All study procedures were approved by the Ethics Committee of Ain Shams University. All included women were interviewed in person using structured questionnaire and informed orally about the procedures and the aim of the study and a written consent was taken to participate in the study. The work is carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

All included women were subjected to the following:

A. Full history taking laying stress on mother's age, full obstetric history, informations about the current use of multivitamin, cigarettes smoking, caffeine intake, dietary micronutrient intake including folate, vit B-12 and vit B6, history of chronic diseases, history of taking medications (e.g. antiepileptic drugs) and time between last pregnancy and participation in the study
B. Complete general examination.
C. Withdrawal of a fasting blood sample by routine venipuncture to measure plasma concentration of:
   - Vitamin B-12 and folic acid.
   - Homocysteine.
   - Methionine.
   - S-adenosylmethionine (SAM)
   - S-adenosylhomocysteine (SAH).

3. Sample preparation

Blood samples were collected into evacuated tubes containing EDTA, immediately chilled on ice, and centrifuged at 4000×*g* for 10 min at 4 °C. Aliquots of the plasma layer were transferred into cryostat tubes and stored at −20 °C until analysis.

3.1. Plasma vitamin B-12 and folic acid concentration

They were measured by using Radio immunoassay kit [Simul TRAC – SNB RadioAssay kit for Vitamin B-12 (^{67}Co) & Folate (^{126}I)] MP Biomedicals [16,17].

3.2. Plasma(Homocysteine – Methionine-SAH-SAM)

Was measured using High-Performance Liquid chromatography (HPLC) [18]. HPLC (Beckman) system Gold, dual pump, Module:125. Kanauer Injector, with a 20 µL loop. Module 166 variable UV detector. HPLC column "phenomenex" (Lichrosorb RP-18; 5 µm, 250 × 4.6 mm, USA).

3.2.1. Development of standard curves

Standard curves of (Homocystiene-Methionine-SAH-SAM) were plotted using four different concentrations (2, 10, 50,

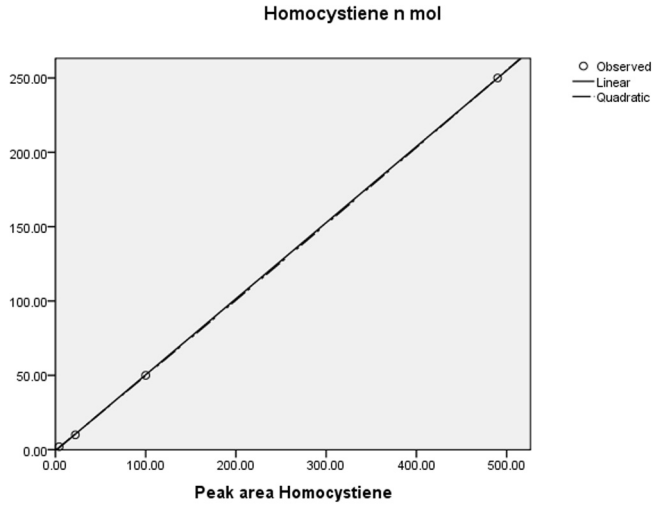

Fig. 1. Standard curve of Homocystiene.