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# DO ABNORMAL LEVELS OF SERUM FOLATE, VITAMINB12, HOMOCYSTEINE IN MATERNAL SERUM SERVE AS A PREDICTIVE MARKER FOR CONGENITAL HEART DEFECTS?

Biochemistry

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## ABSTRACT

Congenital Heart Defects (CHD) is a morphological defect in the structure of heart, which occurs during embryonic stage and is the leading non-infectious cause of mortality and morbidity in newborns. The prevalence of CHD varies widely among worldwide with a prevalence of 9.3 per 1,000 live births in India. There are many causes that results in congenital heart defects which includes genetic and environmental factors. Several biochemical studies demonstrated that raised homocysteine, low folic acid and vitamin B12 levels are the primitive cause for CHD. Furthermore, it is observed that deficiencies in nutrients such as folic acid and VitaminB12 may lead to several birth defects like neural tube defects, growth retardation and elevate the plasma homocysteine levels which have been implicated in the causation of CHDs in foetuses. The present study was aimed to evaluate the effect of homocysteine, folic acid and VitaminB12 levels with CHD in foetuses. A total of 100 women carrying foetuses with CHD along with 100 women carrying normal foetuses were enrolled for the present study. The homocysteine levels were raised in women carrying a foetus with CHD and differed significantly between the groups, while the folic acid was low in both the groups.

Thus, an antenatal test panel comprising of vitamin B12, folic acid and homocysteine level could serve as a predictive marker of CHD risk in foetuses and refined nutritional intervention strategies like folate intake in diet and supplements may reduce the risk of CHD's in foetuses.

## KEYWORDS

Congenital Heart Defects; Folate deficiency; Vitamin B12 deficiency; homocysteinemia; hyperhomocysteinemia

### Introduction

Congenital heart defects (CHDs) is a morphological defect in the structure of the heart which occurs during embryonic stage; are the most common developmental anomalies and the leading non-infectious cause of mortality and morbidity in newborns[1]. The reported incidence of CHD is 4-10/1000 live births[2,3] according to various series from different parts of the world leading to high mortality and morbidity. With a believed incidence rate of 6/1000 live births, nearly 180,000 children are born with CHD each year in India. Approximately 10% of present infant mortality in our country may be accounted for by CHD alone[4]. It results due to genetic, environmental, malnutrition, infections or exposure to harmful pollutants and radiations, maternal alcohol intake and smoking are implicated as causative factors[5]. Advances in our molecular understanding of normal heart development have led to the identification of numerous genes necessary for cardiac morphog enesis. More recently, studies have identified single nucleotide polymorphisms and sub-microscopic copy number abnormalities as having a role in the pathogenesis of congenital heart disease[6].

Over the past decades, a series of clinical and basic research studies showed that the folate metabolism pathway is involved in the development of CHD. Peri-conceptional folic acid supplementation may prevent fetal CHD and folate deficiency in a pregnant woman who potentially contributes to CHD in the developing embryo[7,8]. Additionally, the Vitamin $B_{12}$ or cobalamin is also identified to play an important role in folate metabolism. It in the form of methylcobalamin, participates in the folate-dependent methylation of homocysteine to form methionine in the presence of the enzyme methionine synthase. Any deficiency in vitamin $B_{12}$would result in raised homocysteine and maternal hyper-homocysteinemia, risk factors for CHD[9]. Therefore, the present case control study was aimed to estimate the levels of vitamin $B_{12,}$ Folic acid and homocysteine in women carrying CHD foetuses and also to evaluate their effect on foetuses with different types of CHD[10].

### Materials and Methods

A total of 200 samples comprising 100 pregnant women with CHD fetuses and 100 carrying normal fetuses were enrolled for the present study and were recruited from various hospitals in and around Hyderabad like Govt. Modern Maternity Hospital, Niloufer Pediatric Hospital, Care Hospital and Asian Institute of Fetal Medicine.

The pregnant women were scanned by 3D/4D ultrasound for the fetal anomalies by a fetal medicine specialist and pediatric cardiologist and then enrolled for the study. Pregnant women with known infections such as rubella and any preexisting medical illness such as diabetes, hypertension and women habituated to alcohol or tobacco were excluded. The epidemiological factors such as height, weight, dietary habits along with teratogenic effects of certain drugs, smoking and alcoholic habits, family history of disease (pedigree analysis) and presence of any other disease was recorded in specially designed structured questionnaire.

Blood samples were collected into vacuum tubes without anticoag ulant and with EDTA anticoagulant. The serum and plasma obtained respectively were stored at -80°C till use. The serum sample was used for the estimation of VitaminB12 and folic acid while the plasma was used to estimate the levels of homocysteine in maternal serum of both cases and controlsusing standard kits. Estimation of Vitamin B12 and Folic acid was carried out for all the samples by ELISA kits from Biodetect, India while Homocysteine levels were assayed by analyzer using a kit from Proton, India.

### Results

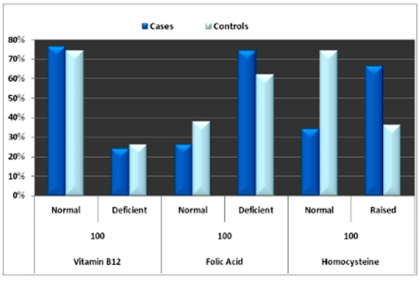
#### Characteristics of the study population

Data analysis of 200 samples revealed that the mean age of samples was ranging between 19 to 37 years. Examination of epidemiological factors showed that consanguinity and a previous history of fetus with CHD were contributing 37% and 9% respectively for having CHD fetuses. Among cases, nearly 26% of women presented the hypothyroidism while 9% were diabetic. Further analysis with respect to the type of CHD, revealed an increased prevalence of VSDs (58%) followed by pulmonary defects (29%) and ASDs (19%).

### Biochemical assays

Of the total 100 cases analyzed, 31% were deficient of VitaminB12, 74% for folic acid while 66% of them were having raised homocysteine levels. Among controls, 26% were deficient of VitaminB12, 62% were deficient for folic acid while 36% were having raised homocysteine levels (**Figure 1**). The plasma homocysteine levels differed significantly between the groups with respect to raised and normal levels (p=0.0001)

**Figure 1**: Distribution of individuals into groups based on the levels of Vitamin B12, Folic acid and Homocysteine




The mean vitamin B12, folic acid and homocysteine levels in cases are 318 ± 133.62, 3.29 ± 2.01 and 18.25 ± 10.66 respectively while they were 315 ± 105, 3.20 ± 1.86 and 16.9 ± 9.01 correspondingly in controls **(Table 1).** The levels did not vary among the groups (p>0.05).

**Table 1:** The mean values of vitaminB12, folic acid and homocysteine levels among cases and controls

| Groups | VitB12 | Folic Acid | Homocysteine |
|---|---|---|---|
| Patients | 318 ± 133.62 | 3.29 ± 2.01 | 18.25 ± 10.66 |
| Controls | 315 ± 105 | 3.20 ± 1.86 | 16.9 ± 9.01 |
| Z Value (p value) | 0.17 (0.85) | 0.32 (0.74) | 0.96 (0.33) |

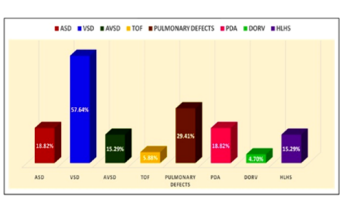
Additionally, for further analysis the congenital heart defects were classified into five defect categories based on anatomical lesion: 1) conotruncal—including transposition of the great arteries, tetralogy of Fallot, truncus arteriosus, double outlet right ventricle, malaligned ventricular septal defect, and interrupted aortic arch type B; 2) septal—including atrial, ventricular, and atrioventricular septal defects; 3) right-sided obstructive—including pulmonary valve stenosis, and pulmonary and tricuspid atresia; 4) left-sided obstructive—including aortic valve stenosis, hypoplastic left heart syndrome and variants, coarctation, and interrupted aortic arch types A and C lesions; and 5) complex cases—combination of three or more of the above cardiac defects [17]. The levels of the biochemical assays performed in these groups are showed in Table 2 and Figure 2.

**Table 2:** Levels of VitB12, Folic acid and Homocysteine in various sub groups

| Groups | Vitamin B12 100 | Folic Acid 100 | Homocysteine 100 |
|---|---|---|---|
| Controls (100) | 315 ± 105 | 3.20 ± 1.86 | 16.9 ± 9.01 |
| Cases (100) | 318 ± 133.62 | 3.29 ± 2.01 | 18.25 ± 10.66 |
| Group1 (15) | 290.68 ± 121.02 | 3.52 ± 2.06 | **25.74 ± 7.16*** |
| Group 2 (34) | 323.27 ± 117.48 | 3.01 ± 1.82 | 16.59 ± 10.16 |
| Group 3 (10) | 312.24 ± 105.34 | 3.08 ± 2.23 | **18.52 ± 10.97** |
| Group 4 (18) | 286.23 ± 126.18 | **2.99 ± 1.79** | **19.79 ± 12.52** |
| Group 5 (23) | 316.29 ± 145.05 | **2.37 ± 2.37** | 16.07 ± 9.66 |

*p<0.0001

**Figure 2: Prevalence of various CHD defects among the cases**




**ASD** = Atrial Septal Defects; **VSD** = Ventricular Septal Defects, **AVSD** = Atrioventricular Septal Defect; **TOF** = Tetralogy Of Fallot; **PULMONARY DEFECTS** = Pul. Atresia AndPul. Stenosis; **PDA** = Patent Ductus Arteriosus, **DORV** = Double Outlet Right Ventricle; **HLHS** = Hypoplastic Left Heart Syndrome

Among the CHD sub groups, the serum homocysteine levels were significantly elevated in group 1 compared to controls (p<0.0001), group 3 and group 4 while the folic acid levels were low in group 4 and 5.

### Discussion

Congenital heart defects results from complex interactions involving environmental exposures, maternal lifestyle factors, and genetic susceptibilities. Several studies had also suggested that multivitamins containing folic acid may protect against congenital heart defects[7,11]. Disturbed/defective folate and homocysteine metabolism in CHDs has been put forward by several studies. But, the data available on its effect on cardiovascular and nervous system in foetuses are scanty. Moreover, there are no studies which have attempted to prove that the defective folate pathway in Indian women could be the major cause for CHD. Hence, the objective of the present study was aimed to estimate the plasma levels of folate, vitB12 and homocysteine, study the influence of these levels with the type of heart defect and to highlight the association between low folic acid deficiency and raised homocysteine.

The present study demonstrated that nearly 66% of cases had greater homocyeteine levels which is much higher compared to the findings of Selhub et al.,[12] and Hobbs etal.,[13] who reported it to be only 34%. No significant difference in vitamin B12 and folic acid levels between cases and controls. The link between folate and CHD could not be established. However, the cases showed higher mean concentration of homocysteine compared to controls. These observations were corresponding with the findings of Hobbs et al., 2005[13] with respect to the levels of vitamin B12 and folic acid, while their study showed lower homocyeteine levels contradictory to our findings. Additional studies had showed increased concentrations of homocysteine in women who have had preeclampsia and recurrent early miscarriages. In a study by Kumar et al., 2014[14] in India demonstrated a raised homocyeteine levels in Down syndrome children with CHDs.

Analysis of biochemical levels among the groups, suggested raised maternal homocysteine as the primary cause for CHD in foetuses, these findings were similar to the observation of Hobbs et al., 2005[13] which indicated that women who had children with CHDs had higher plasma homocysteine levels than controls in Caucasian population. Since there are not many studies pertaining to categorizing CHDs into groups and CHD in foetuses, there was no scope in the current study to precisely compare the observations with clinical traits of others. Several invivo and invitro studies suggested homocysteine as an independent risk factor for coronary artery disease and for every rise of 3umol/L homocysteine there would be 10% risk of cardiovascular events.

Folic acid and VitB12 are the vital regulators in the metabolism of homocysteine. It is derived from methionine by utilizing a methyl group. The homocysteine produced is again remethylated back to methionine by transmethylation. Methyltetrahydrofolic acid, a derivative of folic acid also provides methyl for remethylation of homocysteine. Hence, folic acid deficiency prevents remethylation of homocysteine and also influences the production of MTHF that affects the activity of MTHFR. Furthermore, the possible mechanisms by which homocysteine may have an embryotoxic effect include oxidative stress and secondary accumulation of SAH, which leads to product inhibition of DNA methyltransferase reactions, DNA hypomethylation and altered gene expression[15].

### Conclusion

In conclusion, a pre-conception test panel comprising of vitamin B12, folic acid and homocysteine levels could serve as a predictive marker of congenital heart defect risk in fetuses and refined nutritional intervention strategies like folate intake from diet and supplements may reduce the risk of CHD's in foetuses. Prior folate supplementation for women planning for pregnancy with previous history of CHDs child or fetuses along with MTHFR mutation analysis for dosage sufficiency could be made mandate for the well being of the fetuses.

### Acknowledgement

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**Conflict of interest**
None

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