DYSLIPIDEMIA IN PREGNANCY MAY CONTRIBUTE TO INCREASED RISK OF NEURAL TUBE DEFECTS -A PILOT STUDY IN NORTH INDIAN POPULATION

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ABSTRACT

Neural tube defects are congenital structural abnormalities of the brain and vertebral column resulting from improper or non-timely closure of the neural tube. Prevalence of neural tube defects is reported to be higher among women with diabetes mellitus and obesity. This study was designed to investigate the relation between the presence of dyslipidemia in antenatal patients and the risk of fetal neural tube defects. The present study was an observational, cross-sectional study involving 129 pregnant women in 16 to 18 weeks gestation period. Of these, 80 women had normal pregnancies and 49 were clinically high-risk cases for neural tube defects. Fasting blood samples were analyzed for blood sugar and lipid profile by enzymatic assay and alpha-fetoprotein levels using Enzyme Immunoassay. Alpha-fetoprotein (AFP) values were converted to Multiples of Median (MoM) appropriate for the gestational age. Based on AFP values, women were labeled as screen negative (AFP <2 MoM, n= 102) and screen positive (AFP > 2 MoM, n =27). Screen positive women were further evaluated by ultrasound and 21 women were found to carry a neural tube defects positive pregnancy. Statistical analysis was done on SPSS software. Body weight of the women showed a significant positive correlation with serum triglycerides, plasma sugar and AFP MoM values. A significant difference was observed in serum cholesterol levels ($p=0.038$), triglycerides ($p=0.001$) and plasma sugar levels ($p=0.002$) between normal women and those with neural tube defects positive pregnancy. The Odds ratio for neural tube defects risk in dyslipidemic cases was 24.23 (CI 4.73 – 148.60) with a relative risk of 12.12. Dyslipidemia especially hypertriglyceridemia was found to be significantly associated with fetal neural tube defects.

KEY WORDS

Neural tube defects, Dyslipidemia, Alpha Feto Protein, Multiples of Median.

INTRODUCTION

Neural tube defects (NTD) are congenital structural abnormalities resulting from a defect in any part of the neuraxis. The presentations vary from anencephaly, encephalocele, spina bifida occulta or cystica. They comprise one of the most common and severe congenital malformations (1). The prevalence of NTD from different parts of India has been

reported to vary from 0.5 to 11 per 1000 births (2-4). In general the prevalence of NTD in north Indian states like Punjab, Haryana, Delhi, Rajasthan, UP and Bihar has been much higher (3.9-9.0/1000) compared to eastern, western and southern parts of the country (0.5-2.64/1000) (4,5).

The development of neural tube is a multi-step process strictly controlled by genes and modulated by a host of environmental factors. Despite considerable advances in the understanding of Neural Tube Defects, etiology in many cases still remains unknown. It is widely accepted that interplay of genetic and environmental factors may result in neural tube defects (6). The etiology of NTD is very heterogeneous, with the impact of a given risk factor varying by the types of NTD and the presence or absence of other defects (7). The recurrence risk for NTD is approximately 3-4%, with the risk being slightly higher if the prior infant or fetus had anencephaly (8). However, 95% of

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infants with NTD are born to parents with no family history of the defect, thereby increasing the importance of search for risk factors for development of neural tube defects. The neural tube closes by approximately the 28th day of gestation (9), so any potential exposures suspected to have caused an NTD would have to have occurred within the first month of gestation.

A number of studies have reported maternal age risk for NTD to be U-shaped, i.e., highest among youngest and oldest women, while other studies have found a decreased risk with increasing age (10, 11). Maternal weight has been associated with NTD risk. Obesity has been linked to increased NTD rates (12). However, another study failed to find any association between maternal weight and NTD risk (13). A few studies have indicated an increased risk of NTD in women with diabetes (14), although other studies reported no association between maternal diabetes and NTD (15). Diabetic pregnancies may be related to the occurrence of hyperlipidemias in late phases probably due to increased insulin resistance (16).

Over the last several decades, women carrying a fetus with an open NTD have been found to have elevated maternal serum levels of alpha-fetoprotein (AFP) (17). Prenatal screening of these substances, along with ultrasonography has allowed NTD to be identified in utero. However, in India due to illiteracy, lack of awareness and cost constraints, screening for NTD is not yet included in routine screening. Hence the prevalence of neural tube defects and their resulting economic and social burden still remains high compared to western population. Studies from various birth defects surveillance systems have found that, in regions where elective termination is allowed, prenatal diagnosis and elective termination reduce the birth prevalence of NTD, with the reduction being greater for anencephaly than for spina bifida (10, 11, 18). Thus this study aimed at screening of the prenatal suspected high-risk patients for NTD using maternal serum AFP. Since, obesity, body weight and elevated blood sugar levels are linked to dyslipidemia; we evaluated its association with the risk of development of NTD in fetus.

MATERIALS AND METHODS

A total of 129 pregnant women in 16 to 18 weeks gestation period were selected from the wards and antenatal clinics of Smt Sucheta Kripalani Hospital, New Delhi that is a tertiary care referral hospital for antenatal patients. Of these, 80 women had normal pregnancies. 49 women were suspected high-risk cases for NTD on clinical evaluation. These women either had a family history or previous suspected history of

NTD or had pregnancy complicated by medical disorders like diabetes, or history of drug intake like antiepileptic drugs e.g. valproate or with a history suggestive of nutritional deficiency and recurrent infections. The institutional ethical committee approved the study and the women were included after a written informed consent. Patients with other conditions that may increase AFP levels (hepatitis, cirrhosis, hepatocellular carcinoma and germ cell cancers) were excluded from the study. 5 ml fasting venous blood sample was collected from each patient and divided into three parts. 1.0ml for AFP, 1.0 ml in Sodium Fluoride for plasma glucose and 3.0ml in plain vial for routine biochemical tests. Plasma glucose was estimated by Glucose-oxidase method and lipid profile (serum cholesterol and triglycerides) was done by enzymatic method on Synchron CX9 using kits and calibrators from Randox.

0.5 ml of serum was stored in duplicate at -20°C until analysed for alpha-fetoprotein levels by solid phase Enzyme Linked Immunosorbent Assay using UBI MAGIWEL kit. AFP values obtained for each patient and control sample were converted to Multiples of Median (MoM) appropriate for the gestational age. AFP values less than 2 MoM were considered as screen negative and those more than 2 MoM were considered to be screen positive and further evaluated by ultrasonography. For further evaluation of biochemical and physiological parameters, the women were divided into the NTD positive pregnant cases and the control group (negative screen test and /or negative ultrasound scan). Data are expressed in Mean $\pm$ Standard Error of Mean. Maternal characteristics were compared between groups using a non-paired t-test for continuous variables, a median or Wilcoxon rank-sum test for non-parametric data, and the chi-square test or Fisher's exact test for categorical data. Odds ratio and 95% confidence interval were derived for comparing women with 2 to 3 features of metabolic syndrome with women having none of the features. Means and standard error of mean were computed and compared using an unpaired t-test. Association of NTD risk with physical and biochemical parameters was assessed using Pearson's correlation coefficient. Statistical analysis was carried out using SPSS for windows 12.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

In this study, we screened 129 pregnant women for NTD using Maternal Serum AFP (MSAFP). Out of the total 129 women screened, 27 women had AFP MoM values more than 2 and were considered screen positive. Screen positive women included 21 women from the high risk group and 6 women were from the normal pregnancy group. Out of the 49 women in high-risk pregnancy group, 21 women turned out to be screen

positive and remaining 28 were screen-negative. All of the 27 screen positive women were further evaluated by ultrasonography. Twenty one of these screen positive women carried fetus affected by NTD. On evaluation, 19 out of 21 women were from the earlier classified high-risk pregnancy group and 2 were from the normal pregnancy group. This indicates that even apparently normal pregnant women may be carrying an NTD positive fetus. All the subsequent comparisons were made between these 21 NTD positive pregnant women (cases) and 108 pregnancies without NTD (controls).

No difference was observed in the age and parity of the control group and NTD positive women. The body weight of NTD positive women was apparently higher than that of normal women but the difference was not found to significant. However, a significant difference was seen between blood sugar levels, serum cholesterol and serum triglyceride levels of cases and controls (Table 1). Serum AFP values (expressed as MoM) were significantly associated with age, body weight, blood sugar levels and serum cholesterol and triglyceride levels in all women. Similarly blood sugar values also showed a positive correlation with age, body weight and serum cholesterol levels and highly significant correlation with serum triglyceride levels (Table 2). In women having dyslipidemia, the Odds Ratio for increased risk of NTD was 24.23 (Confidence Interval 4.73 to 148.60) with a relative risk of 12.12.

DISCUSSION

In present study, out of 129 women screened using maternal AFP levels, 27 women had AFP MoM values more than 2. On ultrasound, 21 screen positive women were confirmed to be carrying NTD positive fetus and had AFP MoM levels between 2.15 and 3.12, whereas women with still higher levels of AFP were NTD negative on ultra sonogram. Thus, in these patients

Table 1: Comparison of physical and biochemical parameters between NTD screen negative and screen positive women

	Control group (with normal fetus) (n =108)	NTD positive pregnant Cases (n=21)	p-value
Age (years)	24.67 ± 0.98	25.08 ± 1.12	0.490
Parity	0.96 ± 0.24	1.02 ± 0.21	0.737
BodyWeight (Kilograms)	56.91 ± 1.7	61.24 ± 3.05	0.112
SBP (mmHg)	118.2 ± 2.56	129.5 ± 3.22	0.132
DBP (mmHg)	74.5 ± 2.08	81.06 ± 5.74	0.08
Plasma Glucose (mg/dl)	84.8 ± 2.47	112.50 ± 6.75	0.002
S. Cholesterol (mg/dl)	176.76 ± 5.09	211.4 ± 12.8	0.038
S. Triglycerides (mg/dl)	156.4 ± 12.9	276.0 ± 14.3	0.001
Mean AFP (Designated as MoM values)	1.02 ± 0.11	2.98 ± 0.36	0.000

Values are given in Mean ± SEM. Abbreviations: AFP- Alpha-fetoprotein, SBP- Systolic Blood pressure, DBP-Diastolic Blood Pressure, MoM- Multiple of Median.

there was no relation between occurrence of NTD and presence of very high levels of AFP.

A significant correlation of NTD with maternal age and body weight and very highly significant correlation with blood sugar, serum triglycerides and serum cholesterol levels was observed. Earlier studies indicate an increased risk of NTD affected pregnancies with an increase in pre-pregnancy BMI (19). Though no data on the pre-pregnancy maternal body weight was collected, other studies have shown that second trimester Body Mass Index (BMI) highly correlates with pre-pregnancy BMI (20).

In the present study, maternal body weight showed a significant correlation with blood sugar levels. One of the studies has shown that glucose production increases with maternal body

Table 2: Correlation of physical and biochemical parameters

	Age of women	Body Weight	Plasma Glucose	Serum Cholesterol	Serum triglycerides
AFP levels	r = 0.286 p = 0.041	r = 0.275 p = 0.050	r = 0.393 p = 0.002	r = 0.333 p = 0.010	r = 0.628 p = 0.000
Age of women		r =0.506 p=0.000	r = 0.297 p = 0.027	r = 0.069 p = 0.591	r = 0.267 p = 0.067
Body Weight			r = 0.278 p = 0.042	r = 0.010 p = 0.967	r = 0.336 p = 0.011
Plasma glucose				r = 0.288 p = 0.038	r = 0.486 p = 0.000
Serum cholesterol					r = 0.518 p = 0.000

weight, but peripheral insulin sensitivity tends to decrease resulting in increased plasma sugar levels with increasing body weight (21). Longitudinal studies of glucose tolerance during gestation have shown a progressive increase in nutrient-stimulated insulin responses despite minor deterioration in glucose tolerance, consistent with progressive insulin resistance (22). Changes in hepatic and adipose metabolism alter circulating concentrations of triacylglycerols, fatty acids, cholesterol, and phospholipids. After an initial decrease in the first 8 wk of pregnancy, there is a steady increase in triacylglycerols, fatty acids, cholesterol, lipoproteins, and phospholipids, which may be attributed to higher concentrations of estrogen and a state of relative insulin resistance occurring in pregnancy (23). In this study, the mean serum triglyceride levels in normal pregnancies were towards the higher side of normal range (156.4 ± 12.9 mg/dl), but these values were significantly lower than the mean triglyceride values of the screen positive patients (276.0 ± 14.3 mg/dl; $p = 0.001$).

Cholesterol is used by placenta for steroid synthesis. Fatty acids are used for placental oxidation and membrane formation. Changes in total cholesterol concentration reflect changes in the various lipoprotein fractions. HDL cholesterol increases by 12 weeks of gestation in response to estrogen and remains elevated throughout pregnancy (24). A significant difference in serum cholesterol levels ($p=0.038$) and highly significant difference is plasma glucose ($p=0.002$) and serum triglycerides ($p=0.001$) between the control group and NTD positive women was observed in this study. Though the mean plasma glucose values of NTD positive women were not found to be in the diabetic range but they were still on the higher side of normal range (112.50 ± 6.75 mg/dl). In the present study, 12- fold higher risk of NTD was observed in women having hypertriglyceridemia. Since the presence of increased body weight, dyslipidemia and elevated blood sugar increase the probability of metabolic syndrome in a patient; we propose that Metabolic Syndrome may be associated with the increased risk of NTD (25, 26). Another, recent population based study has indicated 2-fold to 6-fold higher risk for NTD in the presence 1, and 2 or more features of the metabolic syndrome in the mother (27).

A few hypothesis proposed in the recent past indicate the role of elevated blood glucose levels in development of NTD. The early embryo does not have pancreatic function until 7th week of gestation when β -cells are developed. Thus, at the time of neural tube closure (approximately 4 weeks of gestation) embryos could theoretically receive excess glucose from mother and might be unable to regulate excess glucose from

mother (28). Experimental studies have shown that elevated glucose concentrations lead to oxidative stress and embryonic depletion of inositol that is implicated in abnormal closure of neural tube (29). Though there are many reports of the effects of increased maternal plasma glucose levels on development of neural tube defects (14), to the best of our knowledge none of the manuscripts in the literature have shown the association of NTD with hyperlipidemia. In our study serum triglyceride levels had a higher correlation with the risk of neural tube defects as compared to plasma glucose levels, pointing to a probable role of elevated lipids in causation of NTD.

Available data indicate that in diabetic pregnancies, triglyceride concentrations are increased and HDL cholesterol concentrations are decreased with reference to lipoproteins in nondiabetic pregnant women. Thus a transplacental Free Fatty Acid (FFA) gradient exists across the umbilical circulation in the direction of the fetus and is proportional to the maternal FFA concentration. Transfer of unmeasured amounts of triglyceride fatty acids may occur via placental Lipoprotein Lipase (LPL) and be exaggerated in diabetes where LPL declines in adipose tissue but not in placenta (30). These excess free fatty acids may contribute to formation of ceramides, which are known to have apoptotic properties and may potentiate the embryotoxic effects of glucose (31). Thus we conclude that in addition to glucose, it is quite possible that other metabolic fuels like lipids may also cross the placenta and contribute to the adverse intrauterine environment leading to development of neural tube defects. However, further experimental studies are required before any conclusion is drawn from this preliminary observation.

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