Demographic and biochemical risk factors of Down Syndrome in pregnant women: A pilot study in Sylhet division of Bangladesh

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Abstract
Worldwide research in down syndrome have shown that advanced maternal age (>30 years) at conception, lower folate status and/or impaired folate metabolism, maternal weight gain during pregnancy, low socioeconomic conditions, radiation exposure, use of contraceptive pills, and taking tobacco/smoking are major maternal risk factors for giving birth child with DS. However, the occurrence of those factors in pregnant women at advanced age has not yet been investigated in Bangladesh. Therefore, a large scale study was needed to explore the overall scenario in Bangladesh. This pilot cross sectional study was aimed to determine the serum folate and homocysteine status at advanced age pregnant women. We measured serum folate and homocysteine concentrations using the Enzyme-Linked Immunosorbent Assay (ELISA). Out of 70 pregnant women, serum folate level was low (<5 ng/ml) in 13 (18.57%), intermediate (5-6.9 ng/ml) in 8 (11.43%) and normal in 49 (70%) whereas homocysteine concentrations were found low (<5 ng/ml) in all participants. Finally, questionnaire data indicates, low socioeconomic status, overweight during pregnancy, taking contraceptive pills and taking tobacco/smoking was higher percentage in folate-deficient pregnant women than normal serum folate level women. Additionally, women (n=13) with low folate level belongs to low socioeconomic status, overweight, taking contraceptive pills and tobacco/smoking did not give birth child with DS phenotypes. Together, our preliminary findings give an overview of DS associated risk factors in Bangladeshi pregnant women. Large scale study is going on. In next study, we might able to investigate DS risk factors, their associations with serum folate and homocysteine levels and finally with DS phenotypes in child throughout the country.

Keywords: Down syndrome; risk factors; folic acid; homocysteine; pregnant women; Bangladesh.

Abbreviations: DS: Down syndrome; Hcy: Homocysteine; MI: Meiosis-I; MII: Meiosis-II; NTD: Neural Tube Defect; MDS: Mother Down syndrome; ELISA: Enzyme Linked Immunosorbent Assay; 2nd/3rd trimester: Second/Third trimester; ng/ml: Nanogram/Milliliter.
Introduction

Down syndrome (DS) is a common autosomal chromosomal genetic disorder that occurs in about 1 in 700 birth, and main cause of mental retardation and intellectual disability [1-3]. Studies have shown that the main cause of DS is trisomy (3 copies) of human chromosome 21 [4,5]. The compiling data suggests that the extra copy of chromosome 21 in DS is maternal origin (95%) due to error in chromosomal segregation during meiosis of oogenesis [6-10]. Notably, increased maternal age at the time of conception plays as a major risk factor for both Meiosis-I (MI) and Meiosis-II (MII) maternal nondisjunction errors [8,11,12]. Although advanced maternal age is a major risk factor for DS, children with DS are recently born at young age mothers. Different studies reported serum folate and its metabolic genes polymorphisms provably lead full trisomy for chromosome 21 by the aberrant methylation of peri-centromeric regions of chromosome 21 favoring its abnormal segregation during maternal meiosis in pregnant women [13,14]. Some other studies have shown that in addition to increased maternal age, altered folate level and/or metabolism at the time of conception has been associated with chromosomal instability and meiotic nondisjunction suggesting a risk factor for having baby with DS [7,13-18].

Folate or its synthetic form folic acid is a water-soluble organic compound, which belongs to the group of B vitamins. It is an essential micronutrient for the synthesis of RNA and DNA for cell division and tissue growth, a possible contributor in increasing the risk of congenital birth defects, particularly neural tube defect (NTD) [19-22]. Thus, taking folic acid for at least one month before conception reduces the risk of fetal NTD by up to 70% [7]. The active form of folic acid functions as a methyl donor for homocysteine (hcy) remethylation. Moreover, increased level of hcy a metabolic product of folic acid, has been found in mother Down syndrome (MDS) with respect to control mothers [14,23-27].

A significant number of researches have been conducted to know the metabolic and genetic causes of folate status in serum as early maternal risk factors associated with child birth with DS [7,15,16]. However, the prevalence of DS associated risk factors altered folate and homocysteine level in pregnant women in Bangladesh has not been investigated. The main objective of this study was to determine the serum folate and homocysteine level by screening pregnant women and to investigate the major demographic risk factors associated with DS. Finally to investigate DS phenotypes in child born in the mother with demographic and biochemical risk group of DS.

Material and methods

Study design, ethical permission and sample collection

A pilot cross-sectional study was designed as a part of main large scale study. In this study, we recruited pregnant women from Sylhet division, Bangladesh. The ethical permission was taken from intra-departmental ethical committee of Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh. All participants provided written informed consents before enrolling in the study. The confidentiality and rights of the participants were strictly protected.

A structured questionnaire was used to collect socio-demographic data of participants. Total number of study participant was 70 with the age ranged from 30 to 40 years. The monthly income <3000 Bangladeshi taka was considered as low economic conditions and BMI > 25 kg/m² was considered as overweight.

The pediatrician of MAG Osmani medical college hospital evaluates the pregnancy state of all pregnant women enrolled in this study. Our research team explains the objectives of our research and request to enroll in this study to all pregnant women who meet the inclusion criteria. Initially 80 participants were agreed. However, finally from the 80 pregnant women, 70 women are agreed to provide blood. About 4-5 ml of blood was collected from pregnant women at 2³rd/3³rd trimester of their pregnancy following standard venipuncture method.

Serum separation and storage

Blood samples were first allowed to clot for 30 minutes at room temperature and then centrifuged for 10 minutes at 4400 rpm (Thermo Scientific Sorvall S T 8R Centrifuge, USA). The supernatant (i.e serum) was collected and added protease inhibitor (SIGMAFAST™ Protease Inhibitor, Sigma-Aldrich, USA) immediately. Serum samples were stored at -20°C for further analysis.

Measurement of serum folic acid and homocysteine

Serum samples were kept at 4°C for 24 hours prior to analyze. Serum were diluted at 1:2 using sample diluents for both folic acid and homocysteine assay, and then mixed gently. The assessment of folic acid (OKEH02550) and homocysteine (OKEHO2557) was based on competitive enzyme immunoassay and standard sandwich enzyme-linked immunosorbent assay (ELISA), respectively.

The folic acid and homocysteine standard curve was produced by plotting their relative OD450 and standard concentration. The folic acid standard curve is linear and the correlation of coefficient, $R^2$ is 0.9731, and the equation of the standard curve is $y = 0.8415x^{0.571}$. The homocysteine standard curve is also linear and the correlation of coefficient, $R^2$ is 0.9715, and the equation of the standard curve is $y = 0.1021x^{0.7096}$.

Statistical analyses

Statistical analysis was performed in statistical packages for social sciences (SPSS) software.

Results

Serum folate status in pregnant women in Sylhet

Table 1 and 2 shows the serum folate status in pregnant women. Minimum, maximum and mean (arithmetic) values of serum folate concentration were 2.64, 64.67 and 15.92 ng/ml, respectively. We divided the folate status in three groups namely low (<5 ng/ml), intermediate (5-6.9 ng/ml) and normal (≥7 ng/ml). Then we investigate the prevalence of each group in pregnant women with or without folate supplementation. We found low level of folic acid in 13 (18.57%) indeterminate level in 8 (11.43%) and normal in 49 (70%) pregnant women. Moreover, we found 11 (15.62%) folate-deficient pregnant women among 13 those did not take folate supplementation. Intriguingly, we found 2 (15.38%) pregnant women with low level of
folate although they had folate supplementation. In addition, we found 6 (81.82%) and 2 (18.18%) pregnant women with indeterminate level of folate with and without folate supplementation, respectively. We also detected 30 (63.16%) and 19 (36.84%) pregnant women with normal serum folate with and without folate supplementation, respectively (Table 2).

Serum homocysteine level in pregnant women in Sylhet

Serum homocysteine (hcy) level is an indicator of folic acid metabolism state. Accordingly, high level of hcy indicates defect of folic acid metabolism [14]. We measured the homocysteine level in the serum samples to find if the present any defect in folic acid metabolism. We found the low serum homocysteine concentration in all samples compared to the normal value (5 ng/ml), where the minimum value is ~1.53 ng/ml, the maximum value is ~3.06 ng/ml, and the mean value is ~2.48 ng/ml (Table 1).

<table>
<thead>
<tr>
<th>Folate concentration (ng/ml)</th>
<th>Homocysteine concentrations (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (arithmetic)</td>
<td>15.92</td>
</tr>
<tr>
<td>Minimum</td>
<td>2.64</td>
</tr>
<tr>
<td>Maximum</td>
<td>64.67</td>
</tr>
</tbody>
</table>

Table 1: Summary of serum folate and homocysteine concentrations in 70 pregnant women.

Serum folate level No. of PW (%) Folate supplementation Yes [No. of PW (%)] No [No. of PW (%)]

<table>
<thead>
<tr>
<th>Serum folate level</th>
<th>No. of PW (%)</th>
<th>Folate supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;5 ng/ml)</td>
<td>13 (18.57)</td>
<td>2 (15.38)</td>
</tr>
<tr>
<td>Intermediate (5-6.9 ng/ml)</td>
<td>8 (11.43)</td>
<td>6 (81.82)</td>
</tr>
<tr>
<td>Normal (≥7 ng/ml)</td>
<td>49 (70)</td>
<td>30 (63.16)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of serum folate concentrations in pregnant women with or without folate supplementations.

Table 3: Occurrence and associations of major DS associated risk factors in pregnant women with low and normal folate concentrations.

<table>
<thead>
<tr>
<th>Demographic risk factor for DS</th>
<th>Pregnant women with normal folate level (%)</th>
<th>Pregnant women with low folate level (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low socioeconomic condition</td>
<td>50.62</td>
<td>76.92</td>
<td>***p&lt; 0.0005</td>
</tr>
<tr>
<td>Overweight</td>
<td>16.05</td>
<td>38.46</td>
<td>***p&lt; 0.0005</td>
</tr>
<tr>
<td>Taking contraceptive pills</td>
<td>56.79</td>
<td>61.54</td>
<td>***p&lt; 0.0005</td>
</tr>
<tr>
<td>Taking tobacco/smoking</td>
<td>38.68</td>
<td>58.28</td>
<td>***p&lt; 0.0005</td>
</tr>
</tbody>
</table>

Observation of DS phenotypes in child

Thirteen pregnant women, who had low folate level, and from low socioeconomic status, overweight during pregnancy, taking contraceptive pills and taking tobacco/smoking groups was given singleton birth. We investigated DS phenotypes using a questionnaire specific for new born child. In addition, pediatrician also examined the child whether he/she carries any DS phenotypes. However, none of the babies of the folate-deficient pregnant women were found to develop DS phenotypes.

Discussion

Several studies reported that three major mechanisms including non disjunction (95-97%), translocation (3-4%) and mosaicism (1-4%) of chromosome 21 are responsible for DS birth [3,28,29]. Additional studies have also revealed that occurrence of these three mechanisms are favored by having known risk factors like advanced maternal age, impaired folate metabolism, impaired maternal recombination, maternal over weight during pregnancy, low socioeconomic condition, taking contraceptive pills and taking tobacco/smoking leading the prevalence of DS
birth [11,12,15,16,30-34]. Moreover, the prevalence of DS relies on various socio-cultural factors such as frequency of abortion, region, race/ethnicity and gender [35,36].

In the present study, for the first time we investigated the occurrence of socio-demographic (economic status, tobacco eating, overweight) and biochemical risk factors (serum folate and hcy) in pregnant women with >30 years of old (Table 1, 2 and 3). Also, we investigated their association with DS in child. In this pilot study, we did not find any association of socio-demographic and biochemical risk factors with the DS phenotypes of child. However, we found a large percentage of participants were in low-economic, overweight, tobacco taking groups. Notably, serum hcy concentration remains below the normal level during pregnancy that might indicate the normal metabolism of folate [37]. Possible factors for this finding may be the inadequate dietary intake or absorption of folate or more excretion of folate than usual from the body, increased need of folate during pregnancy and unhealthy diet with low socioeconomic and obesity conditions [38-42]. A large numbers of people in Bangladesh are not aware about pregnancy and they do not maintain the nutritional values of daily foods.

There are lots of limitations of the present study. The sample size is very low and participants were from only one divisional city of Bangladesh. However, this is the initial findings of only 70 participants. We are now recruiting participant throughout Bangladesh to investigate the association of demographic and biochemical risk factors of DS. This large scale study might able to state the true associations of maternal risk factors (demographic, anthropometric and biochemical) with DS phenotypes in their child.

Based on the current findings, we might suggest taking folic acid supplement during pregnancy as the requirement of folic acid is much higher during pregnancy and it is hard to fulfill this requirement only from diet. This study might also suggest in avoiding use of oral contraceptive pills, improving socioeconomic conditions, avoiding cigarette smoking or tobacco consumption, creating awareness about DS at individual level, establishing medication center with well trained personnel who will help to reduce the occurrence of birth of child with DS and to improve the lives of people with DS. Moreover, we gently suggest that women with low serum folate level or altered folate metabolism should have prenatal testing for the DS condition and this will help parents and families to prepare for the provision of long-term care of children if he or she is likely to have DS. If a child born with DS, proper anticipatory pediatric management from an early age will help him or her to lead a comparatively independent and healthy life.

Conclusion

This study did not find any DS child born from the mother with low folate levels during pregnancy. Findings warranted further longitudinal study recruiting significant number of pregnant women.

Declarations

Acknowledgements: The team is grateful to Pediatrics department of MAG Osmani Medical college hospital, Sylhet, Bangladesh.

Funding: This research was conducted by the support of SUST research center grant (Grant number LS/2019/07).

References


