INTRODUCTION

Vitamin D is a secosteroid hormone that plays an important role in bone metabolism and mineral homeostasis. Nevertheless, Vitamin D has now increasingly been recognized as a pluripotent regulator of biological functions, namely, cell proliferation, cell differentiation, and modulation of innate and adaptive immunity, in addition to its well-established roles. In the body, Vitamin D is converted to 1,25 dihydroxy vitamin D3 [1,25(OH)₂D], its active form, and it functions by binding with a member of the nuclear receptor superfamily called the Vitamin D...
Receptor. Normal serum concentration of Vitamin D3 in human adults is 60–108 nmol/L (SI Unit) or 25–45 ng/mL. Based on the criteria laid down by the Institution of Medicine and Endocrine Society, serum vitamin D3 levels can be subdivided into three categories, namely, sufficient (>30 ng/mL [75 nmol/L]), insufficient (21–29 ng/mL [50–75 nmol/L]), or deficient (<21 ng/mL [<50 nmol/L]).

The word abortion has been derived from the Latin word Abortio, i.e., “to miscarry.” World Health Organization has defined abortion as pregnancy termination prior to 20 weeks of gestation or with a fetus born weighing <500 g. The incidence of abortion has significant ethnic, geographical, and socio-economic variations. The estimation of the incidence of abortion is difficult, but it varies between 10% and 20% of all pregnancies. About 75% and 80% of all abortions take place before the 16th week and the 12th week of pregnancy, respectively. The etiology of abortion is multifactorial, such as genetic, endocrinial, metabolic, anatomical, infective, immunological, thrombophilia-related, environmental, and finally, unexplained. Maternal factors play a vital role in cases of abortions with chromosomally normal products of conception, and the rate of abortion secondary to maternal causes peaks around 13 weeks of pregnancy. The different maternal risk factors are as follows: Maternal age more than 35 years, infections (5%), endocrine and metabolic factors (10–15%), immunological disorders such as anti-phospholipid antibody (APLA) syndrome due to antiphospholipid antibody-like lupus anticoagulant (LA), anticardiolipin antibody (ACA), Beta-2 Glycoprotein 1 antibody, medical illnesses such as cyanotic heart disease and hemoglobinopathies, cigarette smoking, alcohol intake, radiation (>10 rad), teratogenic agents like antineoplastic drugs, IUD in situ, and finally, unexplained (40–60%).

During pregnancy, low serum Vitamin D level increases the risk of obstetrical complications, which include first-trimester abortion and recurrent abortion. Li et al., have demonstrated a relationship between CD4+ T helper cells (Th) and pregnancy loss. Regulatory T (Treg) cells and T-helper type 17 (Th17) cells are important components of the CD4+ Th system. Treg cells secrete transforming growth factor-b that has an anti-inflammatory role in maintaining fetal-maternal tolerance. On the other hand, Th17 cells can produce interleukin (IL)-17 which has a pro-inflammatory role that has been linked to pregnancy failure. An increase in Th17 cells and a decrease in Treg cells are reported to increase the risk of recurrent spontaneous abortion. IL-23 can promote Th17 cell differentiation and maintain the Th17 response. In a previous study, increased IL-23 expression has been reported in women with recurrent spontaneous abortion. Vitamin D supplementation significantly down-regulates IL-17 expression in cord blood CD4+T cells and thus regulates the abnormal peripheral cellular immunity of recurrent spontaneous abortion patients. Therefore, as a modulator of the immune system, Vitamin D can be considered a regulatory factor in the pathogenesis of abortion.

The guideline proposed by UK Chief Medical Officers and NICE (2012) stated that all pregnant and breastfeeding women should be informed about the importance of vitamin D and should take 10 mg (mcg) of vitamin D supplements daily. However, analytical studies on the role of vitamin D3 in spontaneous abortion are scarce in the state of West Bengal. With this background, a case–control study was conducted to determine the role of Vitamin D in spontaneous abortion cases in relation to normal ongoing pregnancies in a medical college and hospital in Kolkata, West Bengal, India.

Aims and objectives

1. To determine the association between serum Vitamin D3 level and spontaneous abortion in pregnant women attending the Department of Gynecology and Obstetrics in a tertiary medical college in Kolkata
2. To evaluate the outcomes of the study in order to recommend routine vitamin D3 supplementation for pregnant women in the national programme guideline.

MATERIALS AND METHODS

The present study was an analytical case-control study conducted in the Outpatient (OPD) and Inpatient (IPD) of the Department of Gynecology and Obstetrics, Medical College, Kolkata, a government medical college in Kolkata, West Bengal, India. The duration of the study was 1 year (July 2018–June 2019). Ethical approval was duly obtained from the Institutional Ethics Committee (IEC), Medical College, Kolkata, before the commencement of the study (Vide Ref No. MC/Kol/IJC/Non-spon/630/11–2017, Date. September 11, 2017).

Only those cases with gestational age of more than 7 weeks and in whom the ultrasonography (USG) performed before the 7th week of gestation reported fetal cardiac activity were included in the study. The final study population consisted of 50 pregnant women as cases who were admitted in IPD with threatened, incomplete, complete, and missed abortion as detected by clinical examination and confirmed by USG and 50 pregnant women as controls with viable pregnancy of the same gestational age in the same settings confirmed by USG. Exclusion criteria for both groups included those who were already on Vitamin D supplementation, known patients having positive APLA, known patients of hypothyroidism, known patients of...
anatomical uterocervical anomaly and diagnosed case of preconceptional type 2 diabetes mellitus. Investigations performed were USG for confirmation of abortion and viable pregnancy, estimation of serum level of Vitamin D3, measurement of fasting plasma glucose (FPG) and post prandial plasma glucose (PPPG), measurement of serum levels of T3, FT4 and TSH, APLA, LA, ACA IgM and IgG and β2 glycoprotein for both the groups. The controls were matched with cases in terms of age, gestational age, socio-economic status, and laboratory parameters.

The major tool for data collection was a predesigned, pre-tested, structured questionnaire. The questionnaire was pre-tested on 20 pregnant women who were not included in the study population and was modified accordingly. Overall internal reliability (Cronbach's alpha=0.82) of the questionnaire was high. The questionnaire was prepared in English. It was translated into Bengali and then retranslated into English by a language expert. The questionnaire contained baseline socio-demographic information such as age, monthly family income, parity, and period of gestation (POG) in weeks. The other tools used in data collection were OPD prescriptions, bedhead tickets, operative notes, and relevant laboratory and diagnostic imaging reports, including USG reports, reports for FPG, PPPG, T3, FT4, TSH, LA, ACA IgM and IgG, and β2 glycoprotein.

Operational definitions

**Abortion**
It is the premature termination of pregnancy before the period of viability i.e., before 20 weeks of gestation.

**Threatened abortion**
Process of abortion has started but has not progressed to a stage from which recovery is impossible. Clinical features are mild vaginal bleeding and mild lower abdominal pain. Per vaginal (P/V) examination reveals closed external os and uterine size corresponds to period of amenorrhea. Transvaginal sonography (TVS) shows a well-formed gestation ring with central echoes from the embryo, indicating healthy fetus and observation of fetal cardiac motion.

**Incomplete abortion**
The entire products of conception are not expelled, instead a part of it is left inside the uterine cavity. Chief complaints are the history of the expulsion of fleshy mass P/V followed by the continuation of pain abdomen and persistence of vaginal bleeding. P/V examination reveals the uterus smaller than the period of amenorrhea, patulous cervical os admitting tip of finger, and TVS reveals echogenic material within the uterine cavity suggestive of retained products of conception.

**Complete abortion**
Products of conception are expelled en masse. Chief complaints are the history of the expulsion of fleshy mass P/V followed by subsidence of abdominal pain and trace or absent vaginal bleeding.

P/V examination reveals the uterus smaller than the period of amenorrhea, closed cervical os, and scanty bleeding. TVS reveals an empty uterine cavity.

**Missed abortion**
When the fetus is dead and is retained inside the uterus for a variable period. Chief complaints are the persistence of brownish vaginal discharge, subsidence of pregnancy symptoms, cessation of uterine growth, and non-audibility of fetal heart sounds even with Doppler USG. P/V examination shows a firm cervix. Immunological tests for pregnancy become negative. TVS findings are crown rump length and mean sac diameter ≥7 mm without a heart beat and ≥25 mm without an embryo, respectively, and the presence of blighted (anembryonic) ovum.

Categories of serum Vitamin D Level (ng/mL)
- Sufficient: >30
- Insufficient: 21–29
- Deficient: <21.

Ethical considerations
Apart from obtaining approval from IEC, written informed consent was obtained from each participant from each group before the commencement of registration of responses. Anonymity of the participants and confidentiality of data was rigorously maintained. The research followed the guidelines laid down in the Declaration of Helsinki, 1975, updated in 1983 and 2013.

Statistical analysis
Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Version 20.0 (IBM, SPSS Statistics, Chicago, IL, USA). Quantitative data were presented in the form of mean±standard deviation, and categorical data were presented in the form of frequency and percentages. Bivariate analyses were performed using the Chi-square test to determine the difference of the proportion of spontaneous abortion between cases and controls across categories of serum vitamin D level and other categorical variables. An unpaired “t” test was performed to determine significant differences between cases and controls in relation to different quantitative variables, including laboratory parameters. Odds ratios (OR) were calculated to estimate the strength of association between the suspected factors and the outcome. The P<0.05 was considered statistically significant.
RESULTS

A case–control study was conducted in a tertiary care medical college in Kolkata, West Bengal, with the objectives of determining the association between serum vitamin D3 level and spontaneous abortion among 50 women who underwent spontaneous abortion (cases) and 50 women not undergoing spontaneous abortion (controls) in their present pregnancy.

It was observed that the most common type of spontaneous abortion was missed abortion (56.0%), followed by incomplete abortion (28.0%) (Figure 1). The mean ages of the cases and the controls were 24.1±3.27 years and 24.2±3.21 years, respectively, and the difference was not statistically significant (P=0.88). Socio-economic classification was performed according to the Modified BG Prasad Scale, September 2023. Among the cases, 44% belonged to the lower class, followed by lower middle (28.0%). About 22% belonged to middle class, followed by upper (6.0%). Among the controls, 42% belonged to lower class, followed by lower middle (28.0%). No statistically significant association between socio-economic status across the two groups was found (P=0.825, d.f.=1).

The mean POG of cases and controls in weeks were 11.21±2.02 and 11.06±1.85, respectively, and the difference was not statistically significant (P=0.689). The mean FPG of cases was lower than that in controls (84.74±9.64 mg/dl vs. 85.40±10.08 mg/dl) which was not statistically significant (P=0.740). Both cases and controls had almost identical values of mean PPPG (92.58±8.18 mg/dl and 92.98±7.58 mg/dl) with no statistically significant difference (P=0.800). The mean HbA1C of cases was also similar to that of the controls (4.86±0.11% and 4.85±0.11%, respectively) with no statistically significant difference (P=0.590). The mean FT4 level of controls (1.43±0.12 mU/L) was higher than that of cases (1.425±0.13 mU/L). Nevertheless, the difference was not significant statistically (P=0.760). Among cases, the mean TSH was 2.81±0.16 mU/L, whereas the corresponding value among controls was 2.82±0.16 mU/L. The difference was not statistically significant (P=0.860). The mean LA test of cases and controls was 34.92±3.73 seconds and 34.89±3.62 seconds respectively and the difference was not statistically significant (P=0.499). The mean ACA IgM of cases was higher (35.19±2.175 mg/mL) than that of controls (34.72±1.85 mg/mL), and the difference was not statistically significant (P=0.243). The mean ACA IgG of cases (2.5±0.42 mg/mL). And controls (2.5±0.41 mg/mL) were identical and the difference was not statistically significant (P=0.902). Therefore, cases and controls were identical according to matching criteria (Table 1).

Regarding different categories of serum Vitamin D level, 24% of cases (12/50) have been classified as Vitamin D3 deficiency, whereas 44% (22/50) and 32% (16/50) belonged to insufficient and sufficient categories, respectively. Among controls, 10% (5/50) have been classified as deficient, 28% (14/50) had insufficient and 62% (31/50) were found sufficient. The differences in deficiency levels among cases and controls across categories were statistically significant (P=0.009, d.f.=2) (OR: 2.84, 95% CI: 0.92–8.80) (Table 2).

However, the mean serum Vitamin D3 level (ng/mL) of cases and controls were 20.345±10.64 and 21.65±5.91, respectively, the difference being not statistically significant (P=0.450) (Table 1).

Regarding obstetric profile among the two groups, in control, 4% (2/50) mothers had Bleeding Per Vagina (PV), whereas it was present in 50% (25/50) of cases, and the difference was statistically significant (P=0.0001, d.f.=1) (OR: 24.00, 95% CI: 5.25–109.65). Among cases, 46% (23/50) had pain in the abdomen, whereas in controls, 10% (5/50) had abdominal pain and the differences were statistically significant (P=0.0001, d.f.=1) (OR: 7.67, 95% CI: 2.61–22.54). In relation to parity, among cases, 50% of them (25/50) had P1+0, followed by 18% (9/50) having P0+0. In controls, 50% of them (25/50) had P1+0, followed by 20% (10/50) having P0+0. Regarding the history of abortions in previous pregnancies, 16% of cases and 18% of controls had one previous abortion. On the other hand, 16% of cases and 12% of controls underwent two previous abortions. The association of parity between the two groups was not statistically significant (P=0.941) (Table 3).

Figure 1: Distribution of cases according to types of abortion (n=50)
Table 1: Distribution of biochemical parameters in cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case (n=50)</th>
<th>Control (n=50)</th>
<th>t-value (DF=98), P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D3 (ng/mL)</td>
<td>20.345±10.64</td>
<td>21.65±5.91</td>
<td>0.759, 0.450</td>
</tr>
<tr>
<td>POG (week)</td>
<td>11.21±2.02</td>
<td>11.05±1.85</td>
<td>0.402, 0.689</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>84.74±9.64</td>
<td>85.40±10.08</td>
<td>0.333, 0.740</td>
</tr>
<tr>
<td>PP plasma glucose (mg/dL)</td>
<td>92.58±8.18</td>
<td>92.98±7.58</td>
<td>0.254, 0.800</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>4.86±0.11</td>
<td>4.85±0.11</td>
<td>0.540, 0.590</td>
</tr>
<tr>
<td>FT4 (mU/L)</td>
<td>1.425±0.13</td>
<td>1.43±0.12</td>
<td>0.306, 0.760</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>2.81±0.16</td>
<td>2.82±0.16</td>
<td>0.177, 0.860</td>
</tr>
<tr>
<td>LA test (s)</td>
<td>35.49±3.73</td>
<td>34.89±3.62</td>
<td>0.811, 0.499</td>
</tr>
<tr>
<td>ACA IgM (mg/mL)</td>
<td>35.19±2.175</td>
<td>34.71±1.85</td>
<td>1.174, 0.243</td>
</tr>
<tr>
<td>ACA IgG (mg/mL)</td>
<td>2.5±0.42</td>
<td>2.5±0.41</td>
<td>0.169, 0.866</td>
</tr>
<tr>
<td>β2 glycoprotein (mcg/mL)</td>
<td>3.04±0.80</td>
<td>3.06±0.74</td>
<td>0.124, 0.902</td>
</tr>
</tbody>
</table>

Table 2: Distribution of cases and controls according to serum vitamin D3 level

<table>
<thead>
<tr>
<th>Vitamin D3</th>
<th>Case (n=50)</th>
<th>Control (n=50)</th>
<th>Total (n=100)</th>
<th>Chi-square value, P-value</th>
<th>Odds ratio, 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficient</td>
<td>16 (32.0)</td>
<td>31 (62.0)</td>
<td>47 (47.0)</td>
<td>*9.447, (d.f.=2)</td>
<td>2.84 (0.92–8.80)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>22 (44.0)</td>
<td>14 (28.0)</td>
<td>36 (36.0)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>12 (24.0)</td>
<td>5 (10.0)</td>
<td>17 (17.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Obstetric profile of cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case (n=50)</th>
<th>Control (n=50)</th>
<th>Total</th>
<th>Chi-square value, P-value</th>
<th>Odds ratio, 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding per vagina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>25 (50.0)</td>
<td>48 (96.0)</td>
<td>73 (73.0)</td>
<td>*26.839, 0.0001, d.f.=1</td>
<td>24.00 (5.25–109.65)</td>
</tr>
<tr>
<td>Present</td>
<td>25 (50.0)</td>
<td>2 (40.0)</td>
<td>27 (27.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain abdomen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>27 (54.0)</td>
<td>45 (90.0)</td>
<td>72 (72.0)</td>
<td>*14.335, 0.0001, d.f.=1</td>
<td>7.67 (2.61–22.54)</td>
</tr>
<tr>
<td>Present</td>
<td>23 (46.0)</td>
<td>5 (10.0)</td>
<td>28 (28.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P0+0</td>
<td>9 (18.0)</td>
<td>10 (20.0)</td>
<td>19 (19.0)</td>
<td>0.397 (d.f.=3), 0.941</td>
<td></td>
</tr>
<tr>
<td>P1+0</td>
<td>25 (50.0)</td>
<td>25 (50.0)</td>
<td>50 (50.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1+1</td>
<td>8 (16.0)</td>
<td>9 (18.0)</td>
<td>17 (17.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2+2</td>
<td>8 (16.0)</td>
<td>6 (12.0)</td>
<td>14 (14.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The establishment of the immunomodulatory effects of vitamin D3 has suggested that it plays an important role in protecting against spontaneous abortion. It was supported by *ex vivo* analyses demonstrating that Vitamin D3 is able to suppress inflammatory cytokine production by endometrial cells from women with unexplained recurrent spontaneous abortions. In the present study, 24% of cases were found to have vitamin D deficiency, whereas 44% had insufficient and 32% had sufficient levels of vitamin D3. Among controls, 10%, 28%, and 62% have been classified as deficient, insufficient, and sufficient, respectively. The differences in deficiency levels among cases and controls across categories were statistically significant (P=0.009).

The mean serum vitamin D3 level (ng/mL) of cases and controls were 20.345±10.64 and 21.65±5.91, respectively, and neither of the two values belonged to the sufficient category. Similar results have been reported by Hou et al., in a cross-sectional study on 60 women, 30 with a history of successful pregnancy, and 30 with a history of one or more spontaneous first-trimester abortions. Women with successful pregnancy were found to have higher Vitamin D3 levels than women having pregnancy loss (49.32 μg/L vs. 34.49 μg/L, P<0.01). There was a strong association between low vitamin D levels and first-trimester abortion (OR 1.71; 95% CI: 1.2–2.4). Pirdehghan et al., in Iran (2016) reported that the mean serum vitamin D3 level was 20.3±10.8 μg/L in the deficient category findings similar to the present study.

Moreover, 78% of women had less than sufficient levels, similar to the present study, where 68% of cases and 90% of controls had insufficient levels of vitamin D3. Relative risk of abortion was 3.1 (95% CI: 1.39–6.8) and was higher in the severely deficient group. Nevertheless, unlike the findings of the present study, the mean Vitamin D level was significantly higher in...
natural or elective cesarean section in comparison with abortion and emergency cesarean (P=0.040). Dietary practices, outdoor exposure, and quality of antenatal care in different geographical settings may account for such discrepancies. Andersen et al., reported that serum concentrations of vitamin D3 <50 nmol were associated with a >2-fold increase in the first-trimester abortion rate. Ota et al., found that a high proportion of women with recurrent pregnancy loss had Vitamin D3 deficiency and the risk of auto and cellular immune response abnormalities were increased in women with recurrent pregnancy loss and Vitamin D3 deficiency. In a study conducted by Bärebring et al., among 2064 women in Sweden, serum Vitamin D3 sampled before 16th gestational week was found to be negatively associated with abortion and 1 nmol/L increase in it was associated with 1% lower odds of abortion (OR=0.99, P=0.046). Ghaedi et al., found that deficient vitamin D levels (<8 ng/mL) were observed in 33% of women with recurrent abortion in comparison to 15% of women with no abortion in Iran (P<0.05). The present study has found that 16% of cases and 18% of controls had one previous abortion. On the other hand, 16% of cases and 12% of controls underwent two previous abortions. Differences in ethnicity, dietary habits, outdoor habits, and quality of antenatal care may be responsible for such differences in results. In a double-blind randomized clinical trial conducted by Hollis et al., 280 women were divided into four groups: 70 pregnant women with a healthy pregnancy, 70 women with early abortion, 70 non-pregnant women with no history of abortion, and 70 non-pregnant women with a history of abortion. Serum vitamin D3 levels for the four groups were measured as 45.73±2.02 ng/mL, 20.2±1.4 ng/mL, 37.6±2.1 ng/mL, and 11.5±3.1 ng/mL, respectively. An association between serum concentrations of vitamin D3 levels (<50 nmol/L) and abortion and emergency cesarean in comparison to controls was observed. Routine supplementation of Vitamin D3 during first trimester of pregnancy at a safe daily dose of 10 mg should be incorporated into the ongoing Reproductive, Maternal, Neonatal, Child, Adolescent Health Plus Nutrition Programme of our country following the recommendation of UK Chief Medical Officers and NICE guidance (2012). Training of Medical Officers, Auxiliary Nurse Midwives, Accredited Social Health Activists, and Honorary Health Workers should be introduced in order to impart education to all pregnant women along with their family members regarding the importance of Vitamin D3 in pregnancy.

CONCLUSION

Statistically significant Vitamin D3 deficiency was present more in spontaneous abortion cases in comparison to viable pregnancy controls. Moreover, vaginal bleeding and abdominal pain were more common among spontaneous abortion cases than among controls. Routine supplementation of Vitamin D3 during first trimester of pregnancy at a safe daily dose of 10 mg should be incorporated into the ongoing Reproductive, Maternal, Neonatal, Child, Adolescent Health Plus Nutrition Programme of our country following the recommendation of UK Chief Medical Officers and NICE guidance (2012). Training of Medical Officers, Auxiliary Nurse Midwives, Accredited Social Health Activists, and Honorary Health Workers should be introduced in order to impart education to all pregnant women along with their family members regarding the importance of Vitamin D3 in pregnancy.

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REFERENCES

9. Department of Health, Social Services and Public Safety,
Authors Contribution:

CSS- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation; RS- Definition of intellectual content, design, clinical protocol, manuscript preparation, editing, and manuscript revision; DD- Definition of intellectual content, design of study, design, clinical protocol, manuscript preparation, statistical analysis and interpretation; SG- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data analysis, statistical analysis and interpretation, manuscript preparation and submission of article. TKN- Definition of intellectual content, data analysis, design, clinical protocol, manuscript preparation.

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