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Vitamin D and Spontaneous Pregnancy Loss Among Attendants of Al Zahraa University Hospital

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ABSTRACT

Background: Pregnancy loss (PL), the most common negative outcome of pregnancy, has an important emotional impact on women and their partners.

Aim of the work: This work aims to assess serum vitamin D status among pregnant females with and without abortion and to determine possible risk factors influencing vitamin D deficiency among pregnant females.

Participants and Methods: a case control study included250 pregnant women during the first 20 weeks of gestation, aged from 20 to 35 years. Cases comprised those presented with current idiopathic abortion (125), while those without current or previous abortion were selected as controls chosen from attendees of Obstetric and Gynecological Department of Al Zahraa University Hospital in Cairo, Egypt during 2017&2018. The data were collected by completing interview questionnaire, taking anthropometric measurement (weight and height) then calculating body mass index (BMI) and taking blood sampling to assess vitamin D level.

Results: Vitamin D deficiency (VDD) and insufficiency were high among all studied pregnant women (deficient in 57.5% and 60%, insufficient in 32.5% and 27.5% of cases and controls respectively) while optimal level was only among 10% of cases and 12.5% of controls with OR =1.28 and C.I = (0.319- 5.186) . By comparing deficient and optimal groups, it was found that obesity (OR=1.5), nullipara parity (OR=7.77), less indoor activity (OR=1.4) and less outdoor activity (OR=16.47), more fish intake (OR=19) and less yogurt consumption (OR=1.57) were possible risk factors for VDD, while urban residence, no working status, low educational level; low socio economic status, white skin color and exposure to sun with more duration and more body surface exposed were protective factors.

Conclusion: Vitamin D deficiency was more among pregnant females and it was detected as one of the possible modifiable risk factors of spontaneous pregnancy loss especially among recurrent ones.

Keywords: Spontaneous pregnancy loss, Vitamin D.

INTRODUCTION

A pregnancy loss or spontaneous abortion (SA) is defined as the spontaneousdemise of a pregnancy before the fetus reaches viability. Itincludes all pregnancy losses (PL) from the time of conception until24 weeks of gestation [1,2]. Approximately 15% of pregnant womenexperience sporadic loss, 2% experience 2 consecutive PL and 0.4to 1% have 3 consecutive PL [3]. Recurrent pregnancy loss (RPL) is definedas the loss of 2 or more pregnancies; RPL affects approximately 1-2%of reproductivewomen. Causes of RPL are related to genetic factors, anatomical abnormalities, infections, and endocrine disorders [4].

Vitamin D status during pregnancy has been drawing great attention. Today, vitamin D is thought to have multiple functions beyond its role(s) in bone health (due to maintaining calcium homeostasis and promoting bone mineralization [5]. Recently, vitamin D nuclear receptors (VDR) have been identified in numerous tissues including organs involved in the reproduction and infant growth such as the ovary, testis, placenta and mammary gland [8].There is some evidence suggesting that it modulates human reproductive processes[9].Also,it has significant roles in regulating cell proliferation and differentiation and modulating innate and adaptive immune responses [6, 7].It inhibit proliferation of T helper 1 (Th1) cells and limit their production of cytokines, such as interferon gamma,interleukin-2 (IL-2) and tumor necrosis factor-alpha .Conversely, it induces T helper 2 (Th2) cytokines, such as IL-4, IL-5, IL-6.Vitamin D and also regulates B cell immunity. It down-regulates the proliferation and differentiation of B lymphocytes and inhibits IgG production [10]. Thus, a dominant Th2immune response is important to maintain maternal–fetal relationship for successful pregnancy. In contrast autoimmunity and dysregulated cellular immune reactions may be responsible for immunological alterations leading to recurrent pregnancy loss [11].

Vitamin D deficiency during pregnancy is a worldwide epidemic and its prevalence ranges from 18-84%, depending on the country as well as local clothing customs[12].In Egypt, a study conducted in **2015** by **Botros*et al.***[13]found that 54% of

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pregnantfemales had vitamin D deficiency and 10% had vitamin D insufficiencyand another work in **2014** carried out by**El Rifai** ***et al.***[14]reported that the prevalence of vitamin D deficiency and insufficiency among pregnant women reached 40% and 28.9%, respectively.

There are multiple risk factors affecting hypovitaminosis among pregnant women as air pollution, high latitude, seasonal variation more in winter and autumn and the duration of sun exposure, obesity, black skin color as well as the low education level and low income of the mother [15].

Furthermore, maternal VDD had adverse maternal and fetal outcomes, including gestational diabetes, pre-eclampsia, preterm labor, low birth weight, caesarean section and sporadic spontaneous abortion [16, 17].However, the relationship ofvitamin D deficiency and insufficiency in the first-trimesterpregnancy with PL or non-gravid childbearing aged women withspontaneous pregnancy loss history is less clear.

So, the aim of this work was to assess the role of vitamin D and abortion.

STUDY HYPOTHESIS

The null hypothesis (H0) was assumed that there is no difference in vitamin D levels between pregnant females with and without spontaneous PL.

AIM OF THE WORK

The aim of the present work was to determine serum vitamin D status among pregnant females with and without abortion at Al Zahraa University Hospital as well as possible factors affecting its level among pregnant women.

PATIENTS AND METHODS

I. Study Design:

Acase control study was conducted on a total number of 250 participants (125 cases and 125 controls). The study was conducted at Obstetric and Gynecological Department (outpatient and inpatient clinic) of Al Zahraa University Hospital, Cairo during 2017 and 2018.

II. Sampling Technique:

- **Study population and type of sample:**

Two randomly selected days per week were decided for visiting the Obstetric and Gynecological Department, to recruit cases from all pregnant women presented with any type of abortion (missed,threatened) during the first 20 weeks of gestation. Controls were recruited from the pregnant females without either current or previous history of abortion and presented to the department for antenatal care.

Inclusion criteria: include all females during current abortion during the first 20 weeks of gestation, their ages between 20-35 years old with history of regular menstrual cycles and without history of hormonal or vitamin D supplementation within the last 3 months. TheInclusion criteria forselection controlsinclude pregnant women within the same age, during the same gestational age and without current or even previous history of abortion.

Exclusion criteria:

Patients suffering from rheumatoid arthritis or systemic lupus, uterine anomalies, recent acute viral infection, toxoplasmosis and chronic diseases as hypertension, DM, thyroid dysfunction or had multiple pregnancies were excluded.

- **Sample size:**

Sample size was calculated by using Raosoft Sample size calculator with 95% confidence level and by using population size about 1200 and prevalence of spontaneous abortion were 10 %.We have planned the study of matched sets of cases and controls with one matched control per case.All patients were subjected to:

III. Research tools:

1- **Interview Questionnaire:** the structured questionnaire was designed to collect demographic characteristics of the studied pregnant females, Obstetrics and gynecological history as gestational age, parity and history of previous abortion and possible risk factors affecting vitamin D level among pregnant females as sun exposure characteristics, skin color, sun protection use, dietary intake and physical activity.

2- **Anthropometric measurements:** Body weight and height were measured, and then body mass index (BMI) was calculated and according to BMI ***(WHO)*** [18] pregnant females were classified as underweight (BMI= <18.50), normal weight (BMI= 18.50-24.99), overweight (BMI=25.00-29.99) and obesity (BMI ≥30).

3- **Laboratory investigation of vitamin D level:** Subsample (80 participants) was investigated for serum 25-hydroxyvitamin D by using enzyme immunoassay (EIA kit) KT815 with ELISA SystemAS1851Das; Italy (reader) and16041412BioTek; USA (washer) for the quantitative measurement of total 25 OH vitamin D 2/3 level in serum. Vitamin D status was classified according to the endocrine society, the level of serum 25(OH) D was classified into: **Vitamin D**

deficient if serum 25(OH) D was (≤20) ng/dl, **Vitamin D insufficient if** serum 25(OH) was (21-29) ng/dl and **Vitamin D sufficient if** serum 25(OH) was (30- 100) ng/dl.

IV. *Statistical analysis*

Pre-coded data were statistically analyzed using SPSS 20. For descriptive purpose, qualitative data were presented as frequencies and percentages. Mean, standard deviations and ranges were used to describe quantitative numeric variables.

To assess the significance in the observed differences between cases and controls, Pearson Chi square- test (X^2) for independence was used for qualitative data and Fishers Exact test was used instead for cells less than 5. The Student's independent t-test was used for the differences between means of two continuous variables of unpaired group. The significance level was taken at 0.05 and 95% confidence limit. The results were deemed to be statistically significant if the p value (two tallied) was ≤ 0.05. Odds ratios (OR) is the preferred measure of association.

The studied group was classified into two groups to assess factors affecting vitamin D level by comparing two groups who were: vitamin D –deficient andvitamin D- optimal.

V. Ethical consideration:

The ethical committee at Al-Azhar Faculty of Medicine for Girls, approved the research, followed by an ethical permission of Al-Zahraa University Hospital and informed oral consent was obtained from all patients.

RESULTS

The mean age of cases and controls were (27.54 ± 4.92 and 25.58 ± 4.3) years respectively, while regarding age group, those whose age ranged between 19 and 24 years represent 31.2 % of cases and 45.6% of controls while those whose age ranged between 30-35 years formed 40% of cases and 2.4% of controls. Also, 85.6% of cases and 62.4% of controls were residing urban areas and 75.2% of cases and 88.8% of controls were housewives. Those who complete high level of education (university and post grade) were more in cases than in controls (39% in cases compared to 20.8% in controls) while 40.8% of cases complete secondary education in comparison to 53.6% of controls withstatistically significant difference (pvalue ≤0.05).In addition, 62.4% of cases and 66.4% of controls were belonging to middle social class.

As regard body mass index (BMI), overweight females represented 33.2% of cases and 33.6% of controls while obese formed 30.4% of cases and 40% of controls.

Table (1) shows that the mean gestational age was 10.78 weeks ± 4.37 for cases and 19.18 weeks ± 2.42 for controls with statistically significant difference between groups, (P value ≤0.05).Regarding parity, the women who had never given birth assumed nullipara and formed 29.6% of cases and 36.8% of controls while those who gave birth three times and more represent18.4% of cases and 7.2% of controls. Nearly one quarter of cases (25.6%) had a history of previous abortion with 68.7% of them occurring before two months of gestation and those who experienced recurrent abortion (≥2) represented 62.5% of those who reported that they had abortion before, **Figure (1).**

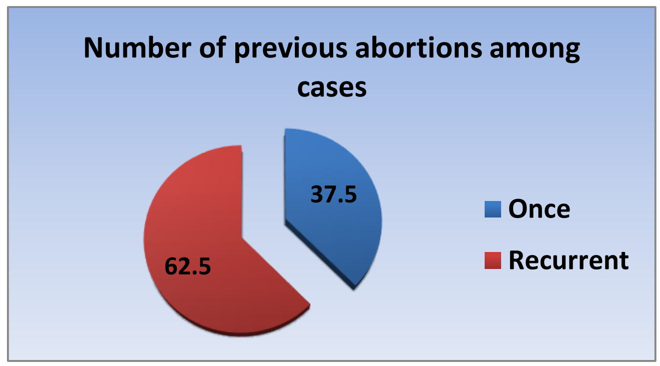

Figure (1): Number of previous abortions among cases.

Table (2) demonstrates that the serum vitamin D level among cases ranged from 6.61 ng/mL to 34.1 ng/mL and its mean ± SD was (19.84ng/mL± 7.04) in comparison to controls whose vitamin D ranged from 11.6 ng/mL to 37.8 ng/mL and its mean ± SD was 20.19 ng/mL ± 7.48.from these figures and according to the ***Endocrine Society cut-offs for serum vitamin D level***, ***vitamin D was*** deficient among 57.5% of cases and 60 % of controls and was insufficient among 32.5% and 27.5% of cases and controls respectively while it was normal among 10% of cases and 12.5% of controls with **OR** =1.28 and **C.I** = (0.319- 5.186) without any statistical significant difference between groups, (P value >0.05).

Table (1):Obstetric history of allparticipants

Items \ Groups	Case (Current Abortion) (125)		Control (Current Pregnancy) (125)		Test of significance
Gestational age:					
○ Range	(3 – 20)		(9- 20)		**t** = 21.293
○ Mean ± SD	10.78 ± 4.37		19.18 ± 2.42		**P** = 0.000*
Parity:	**No =125**	**(%)**	**No =125**	**(%)**	
○ Nullipara	37	29.6	46	36.8	**X^2**=7.288
○ Para 1	36	28.8	39	31.2	**P**= 0.063
○ Para 2	29	23.2	31	24.8	
○ Para ≥ 3	23	18.4	9	7.2	
Previous abortion:					
○ Yes	32	25.6	0	0	
○ No	93	74.4	125	100	
Number of previous abortions:					
○ Once	12	37.5	0	0	
○ Recurrent (≥2)	20	62.5	0	0	
Gestational age of previous abortion:					
○ Before two months.	22	68.7	0	0	
○ After two months.	10	31.3	0	0	

*Statistically significant difference, (P value ≤ 0.05).

Table (3) shows that 87.3% of deficient population and 88.9% of optimal population were housewives with **OR =** 0.861 **C.I:** (0.096-7.719) while those who resided urban areas formed 62% of deficient population and 66.7% of optimal population with **OR =** 0.815 **C.I:** (0.188-3.531). Regarding education, mostly of deficient population (93%) was educated compared to 88.9% of optimal population with **OR =** 0.606 **C.I:** (0.063-5.86). Also, nearly three quarters (73.2%) of deficient population and 88.9% of optimal ones were belonging to low socio-economic status with **OR =**0.342 **C.I:** (0.040-2.92). it is clear that 49.3% of deficient one and 11.1%of optimal group were nullipara with **OR =**7.77 **C.I:** (0.924- 65.46) with statistical significance difference,(pvalue ≤0.05).**Figure (2).**

Table (2):Serum Vitamin D level among studied sub sample of cases and controls and its association with spontaneous pregnancy loss

Groups / Items	Case (Current Abortion)		Control (Current Pregnancy)		Test of significance
	No = 40	**%**	**No = 40**	**%**	
Vitamin D level (ng/mL)					**Fisher's exact**= 0.361
○ Deficiency (≤20)	23	57.5	24	60	**Exact sig. P**= 0.898
○ Insufficient(21-29)	13	32.5	11	27.5	
○ Normal(30-100)	4	10	5	12.5	
Vitamin D level (ng/mL)					**X^2** = 0.125
					OR = 1.28
○ Deficient/insufficient (<30)	36	90	35	87.5	**C.I** = (0.319-5.186)
○ Optimal (≥30)	4	10	5	12.5	**P** = 0.723
Vitamin D level (ng/mL)					**t** = 0.212
○ Mean ± SD	19.84 ± 4.04		20.19 ± 4.48		**P** = 0.833

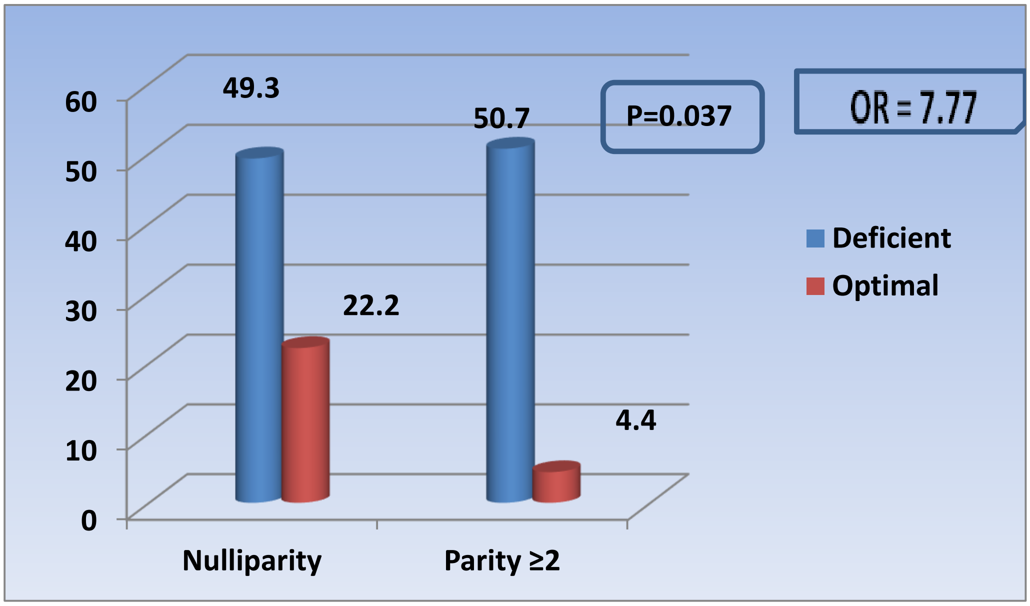

Figure (2): Vitamin D level and parity among studied subsample of cases and controls

Table (3): The association between socio demographic and parity of studied sub sample and vitamin D status.

Items / Groups	Vitamin D level				Test of significance	Exact sig. P-value
	Deficient (≤29 ng/mL)		Optimal (30-100 ng/mL)			
	No= 71	%	No= 9	%		
Occupation: - ○ Housewife ○ Employed	62 9	87.3 12.7	8 1	88.9 11.1	X^2= 0.18 **OR** = 0.861 **C.I:** (0.096-7.719)	1.000
Residence: - ○ Urban ○ Rural	44 27	62 38	6 3	66.7 33.3	X^2= 0.075 **OR** = 0.815 **C.I:** (0.188-3.531)	1.000
Education ○ Low educated ○ High educated	5 66	7 93	1 8	11.1 88.9	X^2= 0.191 **OR** = 0.606 **C.I:** (0.063-5.86)	1.000
Socio Economic Status: - ○ Low ○ High	52 19	73.2 26.8	8 1	88.9 11.1	X^2= 1.043 **OR** = 0.342 **C.I:** (0.040-2.92)	0.437

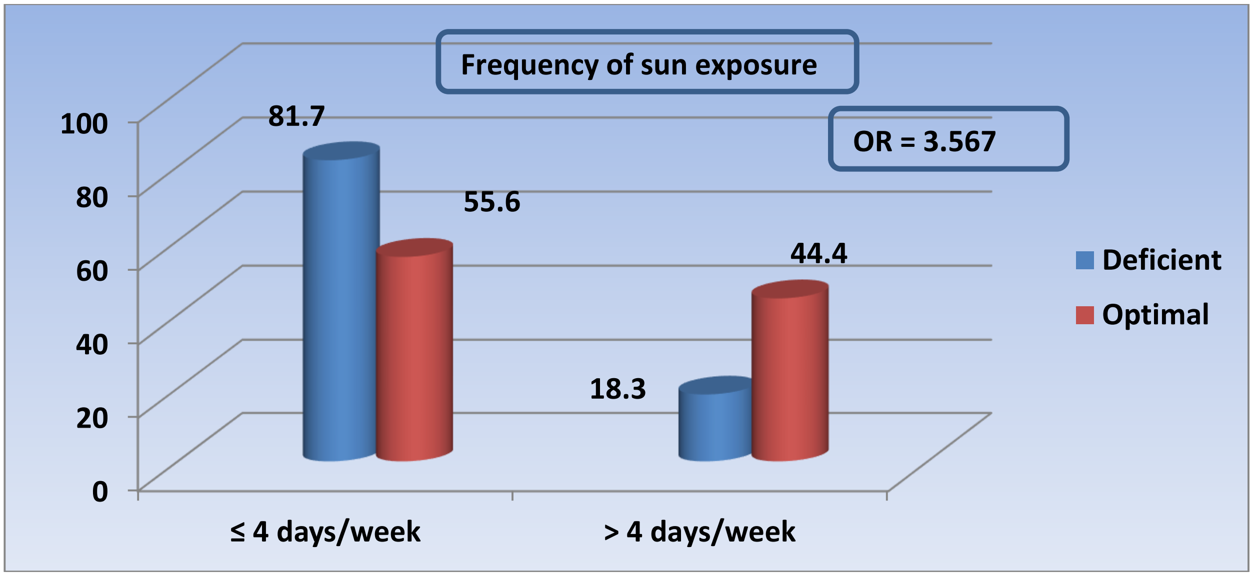

Figure (3): Association of frequency of Sun exposure as regardvitamin Dstatus among studied subsample of cases and controls.

Figure (3) shows that those who exposed to the sun four day and less represented 81.7% for deficient ones and 55.6% for optimal ones with **OR=** 3.567**C.I:** (0.84- 15.15).While **Figure (4)** clarifies that 29.6% of deficient group was obese compare d to 22.2% of optimal ones with **OR** = 1.5 **C.I:** (0.282-7.67).

Table (4): The association between sunning practice of studied sup sample and vitamin D level.

Items / Groups	Vitamin D level				Test of significance	Exact sig. P-value
	Deficient (≤29 ng/mL)		Optimal (30-100 ng/mL)			
	No= 71	%	No= 9	%		
Skin color ○ White ○ Black	29 42	40.8 59.2	4 5	44.4 55.6	X^2= 0.043 **OR** = = 0.863 **C.I:** (0.213-3.491)	1.000
Body parts exposed: ○ Face and hand ○ Hand only	66 5	93 7	9 0	100 0	X^2= 0.676 **OR** == 0.88 **C.I:** (0.809- 0.957)	0.636
Score of Sun Protection Use: ○ 4forms ○ 5forms	44 27	62 38	5 4	55.6 44.4	X^2= 0.139 **OR** = 1.3 **C.I:** (0.322-5.283)	0.729
Time of sun exposure: ○ Around midday (10 am-3pm). ○ Away from midday.	47 24	66.2 33.8	5 4	55.6 44.4	X^2= 0.398 **OR** == 1.567 **C.I:** (0.38- 6.37)	0.712
Adequacy of sun exposure duration: ○ In adequate. ○ Adequate.	35 36	49.3 50.7	5 4	55.6 44.4	X^2= 0.125 **OR** == 0.778 **C.I:** (0.193- 3.137)	1.00

***Significant level (pvalue ≤0.05).**

Table (4) clarifies thatthose who were belonging to black skin color represented 59.2% of deficient group compared to 55.6%of optimal ones with **OR** = 0.863 **C.I**: (0.213-3.491). Concerning to sun exposure, majority of participant exposing face and hand to the sun represented 93% and 100% for deficient and optimal ones respectively with **OR** == 0.88 and **C.I:** (0.809- 0.957). Also, those who exposed to the sun around midday (10 am-

3 pm) represented 66.2% of deficient population compared to 55.6% of optimal ones with **OR ==** 1.567 **C.I:** (0.38- 6.37). Also, this table shows that duration of sun exposure was adequate in 50.7% of deficient ones and 44.4% of optimal group, while 44.4% of optimal ones used 5 forms of sun protection clothing that used outdoor to protect themselves from the sun in comparison with 38% of deficient group.

Table (5):The association between dietary sources of vitamin D among sub studied sample and their Vitamin D level.

Items / Groups	Vitamin D level				Test of significance	Exact sig. P-value
	Deficient (≤29 ng/mL)		**Optimal** (30-100 ng/mL)			
	No= 71	**%**	**No= 9**	**%**		
Milk: -					**X^2**= 0.016 **OR =** 0.915 **C.I:** (0.226-3.696)	1.000
○ ≤ 3 times /week	30	42.3	4	44.4		
○ >3 times /week	41	57.7	5	55.6		
Yogurt: -					**X^2**= 0.292 **OR =** 1.57 **C.I:** (0.302-8.182)	0.456
○ ≤ 3 times /week	22	31	2	22.2		
○ >3 times /week	49	69	7	77.8		
Cheese: -					**X^2**= 0.188 **OR =** 0.724 **C.I:** (0.167-3.132)	0.734
○ ≤ 3 times /week	42	59.2	6	66.7		
○ >3 times /week	29	40.8	3	33.3		
Egg: -					**X^2**= 0.816 **OR =** 0.514 **C.I:** (0.119-2.219)	0.487
○ ≤ 3 times /week	36	50.7	6	66.7		
○ >3 times /week	35	49.3	3	33.3		
Oily Fish:					**X^2**= 12.159 **OR =** 19.048 **C.I:** (2.240-164.9)	0.001*
○ Weekly	50	70.4	1	11.1		
○ Monthly	21	29.6	8	88.9		
Cooked liver:					**X^2**= 2.305 **OR ==** 0.265 **C.I:** (.043- 1.629)	0.176
○ Weekly	5	7	2	22.2		
○ Monthly	66	93	7	77.8		

Significant level (p.value ≤0.05).

Table (5) demonstrates that milk consumption was nearly similar between the two groups with **OR =** 0.915 **C.I:** (0.226-3.696). Also, those who consumed yogurt more than three times per week represented 69% of deficient group compared to 77.8% of optimal ones with **OR =** 1.57 **C.I:** (0.302-8.182). Regarding oily fish consumption, 70.4% of deficient ones consumed once per week compared to 11.1% of optimal ones with **OR =** 19.048 **C.I :**(2.240-164.9) with statistically significant difference (P≤0.05). While, those who consumed cooked liver monthly among deficient ones were 66% compared to 77.8% of optimal group with **OR ==** 0.265 **C.I:** (.043- 1.629).

Table (6) shows thatabout one third of those (33.8%) with vitamin D deficiency had moderate indoor activity compared to all those with optimal vitamin D with **OR** = 1.4 **C.I:** (1.116 -1.694), While 4.2% of deficient participants had moderate outdoor activity versus to 22.2% of optimal ones with **OR** = 6.47**C.I**: (0.920- 45.5), with statistical significant difference between deficient and optimal groups, (pvalue ≤ 0.05).

Table (6): The association between physical activity of studied sub sample and vitamin D status

Items \ Groups	Vitamin D level				Test of significance	Exact sig. P-value
	Deficient (≤29 ng/mL)		Optimal (30-100 ng/mL)			
	No= 71	%	No= 9	%		
Indoor activity: -					X^2= 14.44 **OR** = 1.4 **C.I:** (1.116 -1.694)	0.000*
○ Low	47	66.2	0	0		
○ Moderate	24	33.8	9	100		
Outdoor activity: -					X^2= 4.415 **OR** = 6.476 **C.I:** (0.920- 45.5)	0.036*
○ Low	68	95.8	7	77.8		
○ Moderate	3	4.2	2	22.2		

****Significant level (pvalue ≤0.05).***

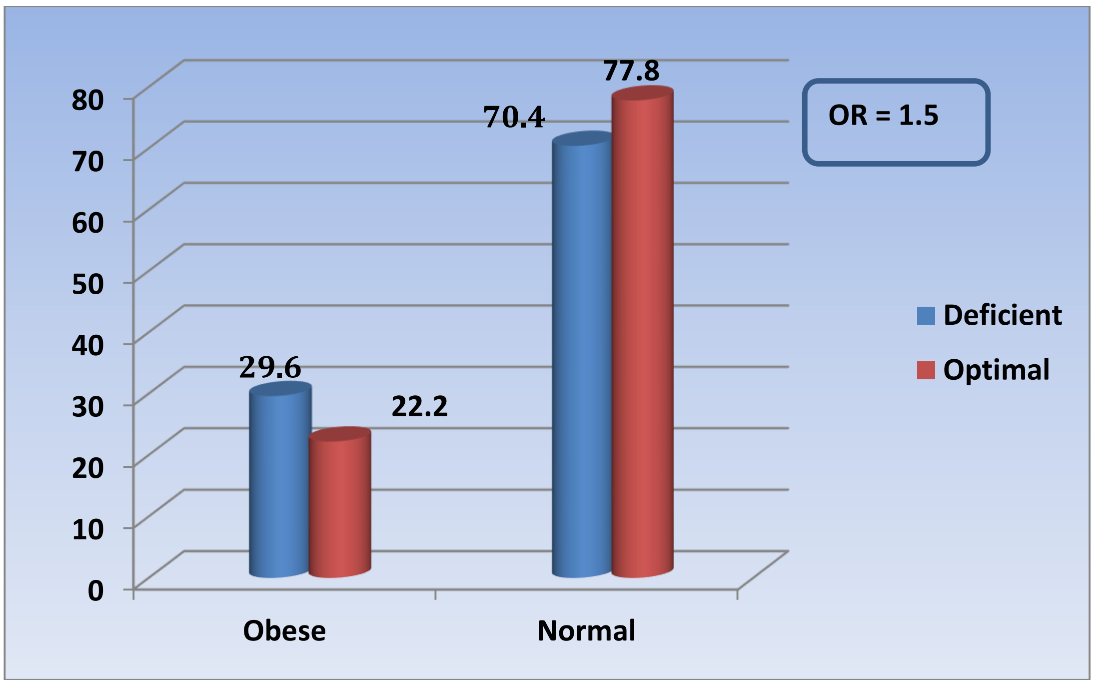

Figure (4):Body Mass Index among studied subsample of cases and controls and vitamin D level.

DISCUSSION

The current study was conducted to delineate the possible relation between vitamin D status and the unfavorable pregnancy outcome, the spontaneous pregnancy loss. Our data revealed that 57.5% of cases and 60% of control were deficient in vitamin D, 32.5% of cases and 27.5% of control had insufficient level, while only10% of cases and 12.5% of control had optimal level of vitamin D. This was in accordance to what was found by ***Botroset al.***[13]who conducted a study in Egypt including healthy females of different age groups in Cairo and Port Said and revealed that 54% of pregnant females had vitamin D deficient and 10% had vitamin D insufficiency.Also,***El Rifai et al.*** [14]reported that theprevalence of vitamin D deficiency and insufficiency among pregnant women were 40% and 28.9%, respectively. Furthermore, ***ElKoumi et al.*** [19] revealed high prevalence of vitamin D deficiency among pregnant females.

In addition, ***Hussein et al.***[20]documented from a study conducted at 12 districts within the UAE, that (69%) of women had vitamin D deficiency, while (22.6%) had vitamin D insufficiency with (8%) subjects with vitamin D status of adequate status. Also, these results were supported by ***Woon et al.*** [21] ***and Aji et al.*** [16].

Regardingthe association ofvitamin D deficiency and spontaneous pregnancy loss, the present study reported that vitamin D deficiency was more among cases (women with current abortion) than control (normal pregnant women) with OR =1.28 and C.I = (0.319- 5.186) which indicated the risk of VDD in developing spontaneous pregnancy loss. These finding agreed with ***Gonçalves et al.*** **[17]**whosystematically reviewed articles studied women with 2 or more spontaneous abortion and its association with VDD and found eleven Studies reported a high prevalence of VD insufficiency (VDI) or VDD in women with recurrent pregnancy loss (RPL). Also, ***Hou et al*****.** **[22]**found thatthe regression analyses showed that PL was significantly inversely correlated with 25(OH) D (P<0.01) and there was a strong association between low vitamin D levels and PL (OR= 1.71; 95% C.I: 1.2–2.4, P0.001).Also***, Ghaedi et al.*****[23]**and ***Andersen et al.*****[24]** supported this association. ***Ozkan et al.*** **[25]** documentedthat the follicular fluid 25(OH) D levels are independent predictors of successful clinical pregnancy following in vitro fertilization; however, serum and follicular fluid levels of 25(OH)D are highly correlated.

On the other hand***, Møller et al*****.** ***[26] and Flood-Nicholset al*****.** **[27]** did not find any association between vitamin D deficiency and the clinical outcome of miscarriage in the first-trimester pregnancy. This difference may be explained not only by differences in study design, but also by differences in our study population relative to other published studies, including the difference in maternal age, sample size and gestational age of specimen collection.

Concerning the possible effect of parity on vitamin D status, the current study revealed that nulliparouswomenwere more among those who were deficient in vitamin D (49.3%) than those with normal level (11.1%) with odds ratio (OR=7.77).These results lie in line with ***.Aji et al*** **. [16]** who found thatpregnantwomen with nulliparous parity status had an eight times higher risk of developing VDD (OR: 7.634, CI 95% 1.550–37.608) (p value = 0.012). Also, **Perez*etal.*** **[28]**Found that nulliparous women (OR: 2.47;p = 0.002) related to deficient 25(OH)D levels. On contrary, ***Andersen et al.*** **[29]**reported that parity was strongly and consistently inversely correlated with vitamin D levels irrespective of season. This difference may be explained as multipara women had multivitamin supplements with previous pregnancies and had experience in nutritional education in comparison with nullipara parity, while ***Woon et al.*** **[21]*****, Ekeroma et al.*** **[30]** ***and El Rifaiet al.*****[14]** showed that there were no associations between parity with vitamin D deficiency.

As vitamin D sources are obtained mainly from three sources, sunlight, diet, and supplementation**, t**he present study found that vitamin D deficiency was more among those with less sun exposure asall optimal group exposes face and hand to the sun with 55.6% of them exposed around midday (between 10 am and 3 pm) compared to 93% and 66.2% of the deficient one. Although the deficient group exposed to the suitable time of sun exposure, they attained low level of vitamin D as they were exposed while they covering all the body except face and hand or hand only and the score of sun protection clothing that used outdoor to protect themselves from the sun (4 forms) were 62% and (5 forms) were 38%.

Moreover, it was documented that nearly one third (31%) of women deficient in vitamin Dconsumed yogurt three times or less per week in comparison to 22.2% of the optimal ones with odds ratio (OR=1.57).In addition, among deficient study population 70.4% of consumed fish weekly compared to 11.1% of the optimal ones with odds ratio (OR=19) with statistical significant difference. It may be due to consumption the type of fish that is poor in vitamin D as fish rich in vitamin D is expensive and not easily available.These results were supported ***by El Rifai et al*** **. [14]** who reported that maternal vitamin D levels showed significant correlations with skin exposure to sun and ***Aji et al.*** **[16]**documentedthat there wasa positive correlation between sun exposure and vitamin D status and most of the women who had less exposure to sunlight were vitamin D deficient.

On the contrary, ***Bukhary et al.*** **[31]** reported that there was no association between sun exposure and vitamin D levels. Furthermore, ***El Rifai et al.*** **[14]** ***and Ekeroma et al.*** **[30]** reported that maternal vitamin D levels were not significantly correlated with eggs and dairy products consumption. This may be due to dietary source acts only by 10% of vitamin D sources.

Our study showed that66.2% of deficient study population were with low level of indoor compared to 0% among optimal ones with odds ratio (OR=1.4) while regarding *outdoor activity*, 95.8% of deficient group were with low level compared to 44.4% of optimal one with odds ratio (OR=6.47). Moreover, The present study revealed that excess body fat, in the form of overweight and obesity is associated with an increased risk of suboptimal vitamin D status (29.6%) of women having deficient vitamin D were overweight and obese compared to (22.2%) of those who had normal level with odds ratio (OR=1.5) without any statistical significant difference.

These results are like the results of ***El Rifai et al.*** **[14]** who reported that there was a significant moderate inverse correlation between BMI and maternal vitamin D level. Also, ***Agarwal, et al***. **[32]** demonstrated that there was a strong correlation between increasing body mass index (BMI) and vitamin D deficiency and insufficiency. All obese females, i.e., BMI ≥30 were found to have vitamin D deficiency (100% prevalence).Moreover, in a Danish study, ***Andersen et al***. **[29]**found that vitamin D insufficiency during early pregnancy was positively correlated with higher pregnancy BMI.In addition,***Perez-Lopezet al.*****[28]**and***Yu et al.*****[33]**supported the current results as they found thatamong pregnant women, maternal vitamin D values during first trimester of pregnancy have been negatively associated with maternal BMI,

On the other hand, ***Woonet al.*** **[21]** ***and Ekeroma et al.*** **[30]** revealed thatthere were no associations between pre-pregnancy BMI with vitamin D deficiency. This difference may be attributed to different ethnic population, sampling size and technique, type of study and study setting.

LIMITATIONS

First, due to high cost of laboratory assessment of vitamin D level, we had a relatively small sample size.Second, some of cases had negative attitude and/or did not show much cooperation to answer the questionnaire.

CONCLUSION

Although Egypt is a country with abundant sunshine all year round, majority of pregnant females had vitamin D deficiency and insufficiency. Vitamin D deficiency had a role in spontaneous abortion especially recurrent spontaneous abortion.

By examining factors influencing vitamin D deficiency among pregnant females during early pregnancy, it was found that urban residence, non-working status, low educational level; low socio economic status, white skin color and exposure to sun with more duration and moreexposed body surface area were protective factors for VDD while obesity, nullipara parity, less indoor and outdoor activity, more fish intake and less yogurt consumption were possible risk factors for VDD.

RECOMMENDATION

On the light of the previous results and discussion we recommend to implement a national strategy for screening, prevention, and treatment of vitamin D deficiency among females in different age group and to increase their awareness about the importance of vitamin D and unfavorable outcome of its deficiency on maternal and child life, ensure antenatal screening for VDD and nutritional education for sources of vitamin D and further research on large sample of population.

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