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Serum Vitamin D among Infertile Men at Basrah Fertility Center and Possible etiological factors

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Abstract: ***Objective:*** Is to evaluate Serum Vitamin D among subfertile men at Basra maternity and children hospital and to evaluate the possible etiology. ***Aim:*** Is to explore the association of serum vitamin D levels with semen parameters and fertility status among males living at Basra city. ***Method:*** A prospective case control study was conducted on a randomly selected 120 infertile male (case group) compared with 120 fertile male that had a child within 1 year from the study time (control group). The study was conducted at Basra fertility center and outpatient department at Basra Maternity hospital during the period from (30th September 2019-30th June 2020). Both groups were investigated through detailed questionnaire and serum level of vitamin D was assessed. Among infertile men, semen samples were analyzed by the same person using the same technique on two occasions one week apart. Serum vitamin D level was measured in both groups using ELISA test. The vitamin 25(OH) D reference ranges that used in this study were as follows: sufficient > 30 ng/mL, insufficient 20-30 ng/mL and deficiency < 20 ng/dL. ***Result:*** The total number of cases was 120, and control was 120.There were a significant relation between serum vitamin D level and subfertility, as lower levels of 25OHD reported among subfertile males who had abnormal semen analysis.The current study revealed that the mean ± SD of serum 25 (OH) D level among cases group was (21.02±8.7) which was significantly lower than its level among fertile male. Most of the (48.3%) cases had deficient serum 25 (OH) D level in comparison to (26.7%) of the control group. This releation was statistically significant using $X2= 29.940$, df= 1, $P<0.05$ S. ***Conclusions:*** There were a significant relation between serum vitamin D level and infertility, as lower levels of 25OHD reported among infertile males who had abnormal semen analysis.

Keywords: Serum vitamin D, malesubfertility, Basra city.

INTRODUCTION:

Infertility is defined by the International Committee for Monitoring is failure to achieve a pregnancy after one year or more of regular unprotected sexual intercourse. According to the World Health Organization , about 10%-25% of the couples complain from infertility disorder. It affects approximately 60-80 million couples world wide. The presence in male and female reproductive tissues of vitamin D metabolizing enzymes and its receptors suggests that vitamin D plays a role in human reproduction (Lorenzen, M. *et al*., 2017). The existence on the head and middle of human sperm of vitamin D (VDR), CYP2R1 , CYP27B1, and CYP24A1 receptors specifiesthat there is some role forvitamin D inthe functionality of sperm, but that function is not fully understood (Blomberg Jensen, M. *et al*., 2010).

In elongated spermatids, epididymis, seminal vesicle and prostate (Blomberg Jensen, M. *et al*., 2010), the VDR and these enzymes are also expressed. In human reproductive physiology, the significance of 25OHD was further examined by demonstrating the presence of the VDR on human sperm and finding a substantial decrease in sperm motility, total number of motile sperm, sex hormone binding globulin quantity, and testosterone / estradiol ration in 25OHD deficient males compared to 25O HD adequate males (Blomberg Jensen, M. *et al*., 2011; & Blomberg Jensen, M. *et al*., 2016). In Iraq , a recent study recorded that nearly half of the studied population had inadequacy and deficiency of 25OHD. In addition, no important association between serum 25 OHD and semen parameters and reproductive hormones in normospermic males was identified , whereas the OAT cohort had a positive relationship with spermmotility (Abbasihormozi, S. *et al*., 2017).

A positive and significant effect of the concentration of 25OHDon sperm motility was identified by Tirabassi *et al.*, (2017). The deficient serum 25OHD levels were related to worse sperm parameters in another study (Ramlau-Hansen, C. H. *et al.*, 2011).The positive correlation between 25 OHD and free testosterone (FT) and total testosterone (TT) levels among healthy men has been reported in several studies (Nimptsch, K. *et al.*, 2012; & Tak, Y. J. *et al.*, 2010) with no significant correlation between 25 OHD and sex hormones reported in one study (10). The relevance of the relationship between serum 25OHD levels and malefertility is clearly inconclusive, but a strong correlation exists (Zhu, C. L. *et al.*, 2016) .

PATIENTS AND METHODS:

A prospective case control study was done at the Basra fertility center, and outpatient department at Basra Hospital for Maternity and Children in Basra city during the period between 1st of February 2020 to 31st of October 2020 . Random sampling of 120 subfertile male with rang of age 18 - 45 years were compared with 120 healthy males of the same age group (control group) with history of child fathering within the last year. In both groups those with a history of chronic illness and endocrine problems, couples with subfertility due to female causes and those with orchitis, cryptorchidism , testicular hypotrophy, testicular trauma, supplementation of 25OHD , treatment with testosterone or thyroxin were excluded. Specially designedquestionnaire regarding Age , BMI, Socio economic class, Occupationwas classified as indoor or outdoor. Type of infertility. Information about diet (vitamin D rich or poor), Good sunlight exposure is for 5-30 min 2-3 times per week.Among infertile men, semen samples were analyzed by the same person using the same technique on two occasions one week apart. Obtaining semen samples by masturbationafter 3-7 days of abstinence from intercourse, analysis was performed using criteria recommended by World Health Organization guideline on sperm analysis (World Health Organization. 2010)..Serum level of Vitamin D was detected spectrophotometrically at 240nm.

SPSS Software version 23.0 along with excel 2010 were used for data entry and analysis. Comparison of results was carried out using chi-square test for categorical dataand Student's t-test for continuous data. P-value of <0.05 was considered statistically significant.

RESULTS:

Table I. The age distribution of males under study.

Age in years	Cases		control		Total	
	No.	%	No.	%	No.	%
≥20	9	7.50%	9	7.50%	18	7.50%
21-40	94	78.30%	102	85.00%	196	81.70%
>40	17	14.20%	9	7.50%	26	10.80%

X2 =2.78, df=2, P value> 0.05.

There was no statistically significant difference regarding the age between cases and controls and the majority were from the age group 21-40 years 94(78.3%) for cases, 102(85%) for control.

Table II. Serum S. 25(OH) D level among fertile and infertile male

S. 25(OH)D level	cases		control	
	No.	%	No.	%
Deficient < 20 ng/ml	58	48.30%	32	26.70%
Insufficient 20-30 ng/ml	29	24.20%	13	10.80%
Sufficient > 30 ng/ml	33	27.50%	75	62.50%

X2= 29.940, df= 1, P <0.05

The percentages of males with deficient 58(48.3%) and insufficient 29(24.2%) serum vitamin D levels were higher among the subfertile (case) group compared to the fertile (control) group 32(26.7%), 13(10.8%) respectively and the vice versa regarding those with normal serum vitamin D level. The differences were statistically significant.

Table III. The distribution of Participants according to the BMI

BMI	Cases		control		Total	
	No.	%	No	%	No.	%
Underweight (<18.5)	4	3.30%	11	9.20%	15	6.30%
Normal (18.5-24.9)	53	44.20%	70	58.30%	123	51.20%
over weight (25-29.9)	45	37.50%	30	25.00%	75	31.30%
Obese(≥30)	18	15.00%	9	7.50%	27	11.30%

X2 =11.61, df=3, P value <0.05 significant

This table shows that the percentage of overweight 45(37.5%), and obese 18(15%) subfertile men were higher compare to fertile men, while those with underweight and normal weight were higher in the control group .These differences were statistically significant. There is no significant differences in BMI between men with low vitamin D and normal vitamin D among fertile group. From the above data, there was no clear effect of BMI on serum Vitamin D level.

Table IV Shows further analysis of the effect of BMI on serum vitamin D level in both cases and controls. About 44(50.6%) of the cases with low vitamin D had normal BMI and about 42(48.3%) of them were overweight and obese. These differences were statistically significant. There is no significant differences in BMI between men with low vitamin D and normal vitamin D among fertile group. From the above there is no clear effect of BMI on serum Vitamin D level.

Table IV. The relationship between BMI and serum vitamin D level

BMI	Cases				Control			
	Low Vitamin D		Normal Vitamin D		Low Vitamin D		Normal Vitamin D	
	No	%	No	%	No	%	No	%
Underweight(<18.5)	1	1.10%	3	9.10%	5	11.10%	6	8.00%
Normal (18.5-24.9)	44	50.60%	9	27.30%	30	66.70%	40	53.30%
over weight (25-29.9)	30	34.50%	15	45.50%	7	15.60%	23	30.70%
Obese(≥30)	12	13.80%	6	18.20%	3	6.70%	6	8.00%
X2,df, P value within cases, and controls	X2=8.543,df=3, 0.036 significant				X2=3.79,df=3 0.285 non significant			
X2,df, P value between cases , and controls regarding Low vitamin D level	X2=12.9,df=3, 0.004 significant							
X2,df, P value between cases, and controls regarding normal vitamin	X2=7.02,df=3, 0.07 non significant							

Table V shows the effect of socioeconomic status on serum vitamin D level. The percentages of men with low socioeconomic status were higher among subfertile men with normal 18(54.5%) and low serum vitamin D level 43(49.4%) compared to those with middle and high socioeconomic status. While in the control group the percentage of men with high socioeconomic status was higher among those with normal vitamin D level 32(42.7%) compared to those with low vitamin D level 14(31.1%), and there is little difference in the percentage of those with middle social class in both men with low and normal vitamin D. These differences were statistically significant.

Table V. The distribution of participants according to the socioeconomic status

Socio-economic state	Cases				Control			
	Low Vitamin D		Normal Vitamin D		Low Vitamin D		Normal Vitamin D	
	No	%	No	%	No	%	No	%
Low	43	49.40%	18	54.50%	9	20.0%	13	17.3%
Middle	30	34.50%	13	39.40%	22	48.9%	30	
High	14	16.10%	2	6.10%	14	31.1%	32	42.7%
X2,df, P value	X2=2.09,df=2, 0.352 significant				X2=1.6,df=2, 0.449 not significant			
X2,df, P value between cases, and controls regarding Low vitamin D level	X2=11.24,df=2, < 0.003 significant							
X2,df, P value between cases and controls regarding normal vitamin D level	X2=20.8,df=2, < 0.0003 significant							

Table VI shows that most of the subfertile males were illiterate or had primary school level of education 77(64.2%), followed by secondary school 32 (26.7%), and higher education 11(9.2%), while in the control group the percentages were 43(36.1%) , 51(42.9%) and 25(21%) respectively, these relationships were statistically significant as shown in table VI.

Table VI. The distribution of participants according to the education level

Education	Cases		Control		Total	
	No	%	No	%	No	%
Illiterate & primary school education	77	64.20%	43	36.10%	120	50.20%
Secondary school	32	26.70%	51	42.90%	83	34.70%
Higher education	11	9.20%	25	21.00%	36	15.10%

X2 =19.423, df=2, P value <0.05 significant

Table VII. The distribution of participant according to the type smoking and vitamin D level

Smoking	Cases				Control			
	Low Vitamin D		Normal Vitamin D		Low Vitamin D		Normal Vitamin D	
	No	%	No	%	No	%	No	%
Smoker	53	60.9%	23	69.7%	20	44.4%	33	44%
Non smoker	34	39.1%	10	30.3%	25	55.6%	42	56%
X2,df, P value	X2=0.79,df=1, P value = 0.37 not significant				X2=0.002,df=1, P value = 0.96 not significant			
X2,df, P value between cases, and controls regarding Low vitamin D level	X2=3.25,df=1, P value = 0.07 not significant							
X2,df, P value between cases, and controls regarding normal vitamin D level	X2=6.06,df=1, P value = 0.013 significant							

The percentages of smokers were higher in infertile group among men with both normal 23(69.7%), and low vitamin D level 53(60.9%), and vice versa among the fertile group. This mean that smoking affect the fertility status rather than S. vitamin D level.

Table VIII. The relation between vitamin D level and sun exposure

sun exposure	Cases				Control			
	Low Vitamin D		Normal Vitamin D		Low Vitamin D		Normal Vitamin D	
	No	%	No	%	No	%	No	%
Good exposure	19	21.80%	32	97.00%	0	0.00%	73	97.30%
poor exposure	68	78.20%	1	3.00%	45	100.%	2	2.70%
X2,df, P value	X2=55.26,df=1, P value = 0.0001 significant				X2=111.8,df=1, P value = 0.0001 significant			

This table (VIII) shows that in both groups the percentages of males with poor sun exposure were higher among those with low vitamin D level 68(78.2%), than those normal vitamin D level 32(97%), and vice versa regarding those with good exposure to sunlight. The associations were significant.

Table IX. The distribution of study sample according to occupation

Occupation	case				Control			
	Low Vitamin D		Normal Vitamin D		Low Vitamin D		Normal Vitamin D	
	No	%	No	%	No	%	No	%
Indoor	67	77%	12	36.4%	9	20%	29	38.7%
Outdoor	20	23%	21	63.6%	36	80%	46	61.3%
X2,df, P value within cases, and controls	X2=17.5,df=1, P value = 0.001 significant				X2=4.5,df=1, P value = 0.003 significant			
X2,df, P value between cases, and controls regarding Low vitamin D level	X2=40.5,df=1, P value = 0.001 significant							
X2,df, P value between cases, and controls regarding normal vitamin D level	X2=0.05,df=1, P value = 0.8 nonsignificant							

This table shows that among infertile male the percentage of males with indoor occupation n was higher among those with low S. vitamin D level 67(77%) and the percentage of those with outdoor occupation was higher among those with normal vitamin D level 21(63.6%) and the relationship were statisticallysignificant. Among the control group the same relationship was foundamong thosewith normal S. vitamin D leveland this relationshipwas also statisticallysignificant. The infertile male with low vitamin D had significantly higher percentage of indoor occupation 67(77%) than those fertile males with low vitamin D level 9(20%).

Table X. The relation between Serum 25(OH)D level and semen analysis results among infertile group

Type of semen abnormality	Oligospermia		Asthino-zoospermia		Teratozoo Spermia		normal	
Vitamin D level	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Low Vitamin D	**58**	**74.4%**	25	69.4%	4	80%	**0**	**0%**
Normal Vitamin D	**20**	**25.6%**	**11**	30.6%	**1**	20%	**1**	100%

Likelihood Ratio, X2 =3.08, df=3, P value = 0.37 not significant.

This table shows the type of semen abnormality among infertile males. The percentages of oligospermia, asthinozoospermia and azoospermia were higher among those with low S. vitamin D 58(74.4%), 25(69.4%), 4(80%), respectively, and Teratozospermia constitute the higher percentage. Only one infertile male has normal semen analysis and it was found that he has normal Vitamin D level also.

Table XI The relation between vitamin D level and Diet among cases and controls.

Type of diet	Cases				Control			
	Low Vitamin D		Normal vitamin D		Low Vitamin D		Normal vitamin D	
	No	%	No	%	No	%	No	%
Diet poor in Vit. D	54	62.10%	3	9.10%	33	73.30%	5	6.70%
Diet rich in vit. D	33	37.90%	30	90.90%	12	26.70%	70	93.30%
X2,df, P value within cases, and controls	X2=9.45,df=1, P value =0.002 significant				X2=8.98,df=1, P value =0.003 significant			

This table shows a clearly significant effect of vitamin D rich diet on serum level of vitamin D. In both groups the percentages of males with poor vitamin D diet were higher among those with low Vitamin D 54 (62.1%), than those with normal vitamin D level 3 (9.1%), and vice versa regarding those with normal S. vitamin D.

DISCUSSION:

In the current study, there was no statistically significant difference regarding the age between cases and controls and the majority were from the age group 21-40 years (78.3%) for cases, (85%) for control. It was noticed that the percentages of males with deficient 58(48.3%) and insufficient 29(24.2%) serum vitamin D levels were higher among the subfertile (case) group compared to the fertile (control) group (26.7%), (10.8%) respectively. Multiple studies showed the same findings, that there is a significant effect of vitamin D deficiency on the male infertility as shown by several studies (Alzoubi, A. *et al*., 2017; & Akhavizadegan, H., & Karbakhsh, M. 2017). The same finding was reported in china by Yu S *et al*., the prevalence of vitamin D deficiency, insufficiency, was found to be 55.9%, and 38.7%, respectively (Yu, S. *et al*., 2015).

Regarding the possible etiological factors, we found that low serum vitamin D level was more common among subfertile men with low socioeconomic status in comparison to the control group where the normal serum vitamin D level was more common among subfertile men with high socioeconomic status. This is goes in accordance with Anwar Ali Jamali who found that dominant ratio of lower economic group (91%), while (6%), (3%) belonged middle and upper group respectively (Jamali, A. A. *et al*., 2018; & Al-Assadi, A. F. *et al*., 2018) . This may be explained by the fact that low socioeconomic situation affect the availability, quality and adequacy of diet rich with vitamin D. In addition to different diseases that affect poor people.

In the current study, most of the subfertile males were illiterate or had primary school education (64.2%), followed by secondary school (26.7%), and higher education (9.2%). This may be explained by that the illiterate people are more prone to different vitamin deficiencies including vitamin D because of poor health awareness and education. In addition to the fact that an association between low level of education with low socioeconomic status was present. This is similar to that reported by Guixiang Zhao *et al*., who found that the higher educational level was associated with decrease the likelihood of vitamin D deficiency (Zhao, G. *et al*., 2012). We also found that smoking affect the fertility status rather than serum vitamin D level. Harlev A *et al*., found that the data on smoking and male fertility reinforce the preferred preventive approach of discouraging smoking and eliminating exposure to tobacco smoke among both males and females in general, and in particular, while trying to conceive (Harlev, A. *et al*., 2015; & Dai, J. B. *et al*., 2015).

This study showed that in both groups the percentages of males with poor sun exposure were higher among those with low vitamin D level and vice versa regarding those with good exposure to sun light in significant relationship. This is supported by a study done by Sollis SS, who estimated that around 5-30 min of sun exposure without use of sunscreen two times a week between 10:00 am to 3:00 pm is enough to maintain sufficient vitamin D level within normal reading (Sollis, S.S. 2015). Pettifor JM reported that decreased sun exposure is a major factor in the development of vitamin D deficiency (Pettifor, J. M. 2014). Holick MF documented that the activation of inactive vitamin D in the skin by ultraviolet B radiation, through sun exposure (Holick, M. F. 2007). Approximately, 80-90% of vitamin D3 derives from sunlight-induced production in the skin, while a small quantity is resultant from diet and supplements (Holick, M. F. 2007; & Henry, H. L. 2011). Getting enough UV rays is the key to the health of male sperm, according to research presented to the Fertility Society of Australia conference in 2008, Laura Thomson, found that vitamin D deficiency caused by too little sun exposure played a significant role in determining male fertility. A group of 123 of the vitamin D deficient men in the Australian study were followed for 3 months as they took vitamin D containing multivitamin supplements, antioxidants and lost weight. Follow-up tests showed improvements in sperm shape and a 75% drop in sperm DNA fragmentation, when the DNA is damaged. In a 25% of these men, pregnancy was successful (Henry, H. L. 2011; & https://www.hippocraticpost.com).

This study shows that among infertile male the percentage of males with indoor occupation was higher among those with low serum vitamin D level and the percentage of those with outdoor occupation was higher among those with normal vitamin D level and the relationship were statistically significant. Among the control group the same relationship was found among those with normal serum vitamin D level, and this relationship was also statistically significant. The subfertile male with low serum vitamin D level had significantly higher percentage of indoor occupation 67(77%) than those fertile males with low serum vitamin D level 9(22 %). Sowah, D., Fan, X., Dennett, L. *et al*.,. Found that, indoor workers significantly had lower 25-hydroxyvitamin D (25-(OH) D) levels compared to outdoor workers (40.6 ± 13.3 vs. 66.7 ± 16.7 nmol/L; $p < 0.0001$) (Sowah, D. *et al*., 2017). This could be explained by those with outdoor occupation had more sun exposure which supply the body with 8-90% of the needed active vitamin D, also some literature related the physical activity with vitamin D concentration. And the outdoor occupations usually combined with hard physical activity (Al-Othman, A. *et al*., 2012; & Freedman, D. M. *et al*., 2013).

It was found that the percentages of oligospermia, Asthinozoospermia and azoospermia were higher among those with low serum vitamin D and Teratozospermia constitute the higher percentage. This is similar to that found by Rehman R *et al*., who found that the 25OHD levels were significantly high in males with normal sperm parameters; 80.90 ± 23.33 nmol/L vs. altered sperm parameter/s, 64.68 ± 24.21 nmol/L (mean ± SD). This may help target health promotion and preventive efforts. Additional 2 studies carried out by Blomberg Jensen Metal, they found the same finding of decreased serum vitamin D level association with abnormal semen parameters (Blomberg Jensen, M. *et al*., 2016b; Blomberg Jensen, M. *et al*., 2012). A contradictory result was found by Hammoud AO, *et al*., Ramlau-Hansen CH *et al*., and Fu L, *et al*., stated that the differences may be related to the difference in study methodologies and sample selection criteria as in this study the control were healthy personals. Nevertheless, vitamin D receptors are found in male reproductive tissues such as human testis, ejaculatory tract, and mature spermatozoa (2), and this indicates a role of 25OHD in human reproduction (Hammoud, A. O. *et al*., 2012; & Fu, L. *et al*., 2017). Recently, Fu *et al*.,. stipulate that 25OHD deficiency reduces testicular weight and sperm quality; moreover, there is suppression of testicular germ cell production and a decline in mature somniferous tubules percentage in mice. They also reported that 25OHD deficiency reduces the enzymes for testosterone synthesis and, consequently, serum, and testicular testosterone levels were decreased (Fu, L. *et al*., 2017).

CONCLUSION:

Low vitamin D is a significant statistically factor for male infertility. Low socioeconomic class, low level education, poor sun exposure and indoor occupations, are among significant causes of serum vitamin D deficiencies.

Recommendations

1. Large prospective studies should be conducted to study the effect of vitamin D supplementation on improvement in semen parameters among infertile males.
2. Screening all infertile males for vitamin D level.
3. The addition of vitamin D supplementation to treatment protocol for infertile males.

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