Original Research Article

A study of vitamin d and thyroid profile to evaluate infertility in females

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Abstract

Aim & Objective: To study the serum levels of vitamin D and thyroid profile to evaluate infertility in females. **Methodology:** The present study was done at Gandhi Hospital, Secunderabad, after approval by Institutional ethical committee of Gandhi Hospital, Secunderabad. All infertility patients with history of not conceiving after 12 months of regular intercourse, without any comorbidities like Hypertension, Diabetes, Heart diseases attending infertility outpatient of Gandhi hospital were enrolled in this study after taking consent. This study was conducted on 50 infertile female cases and 100 fertile controls, in which serum vitamin D and thyroid profile is assayed in Siemens Advia centaur xpt autoanalyzer based on chemiluminescence technique. **Results:** In the present study it was found that 48% of infertility cases belong to age group 32-39 years with mean±SD of age in infertility cases was 30.06±6.34 and controls was 28.6±6.31. In the present study distribution of vitamin D deficiency was mild 20%, moderate 32% and severe 22% in infertile cases, whereas distribution of vitamin D deficiency in fertile females was mild 33%, moderate 6%, severe 2% indicating majority of moderate and severe vitamin D deficiency belongs to cases. vitamin D mean ±SD of controls was 20.5±6.43 with highly significant p value <0.01 showing vitamin D deficiency is common in infertility cases. In this study distribution of vitamin D deficiency is more prevalent in infertile cases 74% compare to 41% of controls suggest vitamin D role in reproduction. In present study mean±SD of T_3 0.74±0.53 in cases, 0.95±0.24 in controls, mean ±SD T_4 is 5.06±3.56 in cases ,6.43±2.04 in controls, mean ± SD of TSH is 6.40±4.02 in cases ,3.68±1.02 in controls with significant p value<0.05.Thus indicating altered thyroid profile can result in infertility. Hypothyroidism constitute about 50% of infertility cases, hyperthyroidism was 8% and euthyroid was 42%. Vitamin D deficiency is more commonly seen in hypothyroid cases 91.6%. **Conclusion:** This study shows negative correlation between vitamin D and TSH levels in infertility which need to be further evaluated. Hence all patients of female infertility should be screened for vitamin D and thyroid profile assessment.

Keywords: Thyroid, Vitamin D, Infertility, TSH.

Introduction

Infertility is inability to conceive after 12 months of frequent coitus[1]. It is multicomplex disorder which has profound medical, psychosocial and economical effect[2]. Infertility is a public health issue which varies widely in different communities according to the prevalence of the condition and its importance in society[3]. The overall prevalence of infertility is 12 -14%. Based on etiology infertility can be classify as male infertility (30%), female infertility (35%), combination of both (20%) and unexplained infertility (15%)[4]. Female infertility can occur due to various causes such as anovulation, hormonal imbalance, sexually transmitted diseases, advance age, reproductive tract diseases, Medical conditions like Hypertension, Diabetes etc., smoking, alcoholism, exposure to radiation during chemotherapy, toxic fumes of leadand Idiopathic[5].

Vitamin D, 1, 25 Dihydroxycholecalciferol is a fat soluble vitamin synthesized in the skin from 7-dehydrocholesterol by the action of ultraviolet radiation of UVB range 290-320nm hence regarded as "sunshine vitamin". It plays a crucial role in various cellular functions like cell differentiation, apoptosis, immunosuppression, decreased proliferation and reduced inflammation. Its mechanism of action of action is mediated through vitamin D receptor VDR, which is expressed in various reproductive organs like ovary, placenta, and uterus[6]. Vitamin D plays an important role in female reproduction, it stimulate progesterone production by 13%, estradiol production by 9%, and estrone production by 21% in ovarian tissue, regulates human chorionic gonadotropin expression and secretion inhuman syncytiotrophoblast and increase placental sex steroidal production, regulates HOXA10 expression in endometrial tissue cell which is important for development of uterus and uterine receptivity to implantation[2]. it has direct effect on Antimullerian hormone (AMH) production, which is responsible for longer maintenance of ovarian reserve[7]. In Polycystic ovarian syndrome (PCOS) through gene transcription and hormonal modulation it influences insulin metabolism[8].

Endocrines plays very crucial role in maintaining fertility. Thyroid hormones have profound effect on reproduction and maintaining pregnancy. Thyroid dysfunctions is considered as an important part for evaluation of infertility in females because they are associated with menstrual disturbances, anovulatory cycles, decreased fecundity and increased morbidity during pregnancy. Higher prevalence of hypothyroidism in infertility is due to altered peripheral oestrogen metabolism, hyperprolactinemia and disturbances in gonadotropin releasing hormone GnRH secretion that result in an abnormal pulsatile release of LH. Thus Proper management of thyroid

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disorders results in achieve maximum fertilization rate, role in oocyte physiology, normalise prolactin levels, normal LH response to LHRH, reduce menstrual disturbances and increases the chance of spontaneous fertility[9,10].

Vitamin D deficiency and thyroid insufficiency both play contributing role in infertility by acting through similar receptor binding site RXR known as steroid receptor alters the immune-regulatory and anti-inflammatory action[11]. Thus both vitamin D deficiency and thyroid disorders can play contributing role in infertility. Assessment of serum vitamin D and thyroid hormone levels help in early diagnosis and treatment of Vitamin D deficiency and thyroid disorders, which increases the chances of conception in all infertility cases, hence the study was undertaken to establish relationship between vitamin D and thyroid hormones inevaluating infertility.

Aim and objectives

Aim

To study the serum levels of vitamin D and thyroid profile to evaluate infertilityin females.

Objectives

1. To measure the serum levels of vitamin D and thyroid profile in infertilewomen age between 18-39 years.
2. To compare vitamin D levels and thyroid profile between fertile and infertilewomen.
3. To identify vitamin D deficiency in infertile women which may act aspredisposing factor for infertility.

Material and methods

Study center

The present study was done at Gandhi Hospital, Secunderabad, after approval by Institutional ethical committee of Gandhi Hospital, Secunderabad. All infertility patients with history of not conceiving after 12 months of regular intercourse, without any comorbidities like Hypertension, Diabetes, Heart diseases attending infertility outpatient of Gandhi hospital were enrolled in this study after taking consent.

Blood samples of infertile cases and fertile healthy women was taken as controls were collected for measuring serum vitamin D and thyroid hormone T3 ,T4 and TSH after taking consent analyzed at central diagnostic laboratory ,Gandhi Hospital, Secunderabad.

Study period

1 Year from October 2018 to September 2019

Study type

Observational Descriptive Study

Sample size

Total sample 150

50 Cases of infertile women.

100 Controls of fertile women.

Inclusion criteria

1. Female patients age between 18-39 years who are not able to conceive sincelast 12 months after regular intercourse.
2. Known cases of infertility without any other medical issues like Hypertension, Diabetes Mellitus, Coronary artery disease or any other systemic disorders.

Exclusion criteria

1. Female infertility cases with structural abnormality of reproductive organs like uterus, fallopian tube etc. example fibroid, septate uterus, polyps.
2. Female age <18 and >39.

Sample collection and storage

After about 8-10 hours fasting 5ml of venous blood was drawn from the infertility cases and fertile controls. The sample was centrifuged at 3000 rpm for 10 minutes and serum was separated and stored in deep freezer 20^0 centigrade until processed. Serum samples were allowed to clot for not more than 30 minutes before centrifugation and separation of serum. The separated samples were analyzed in batches in siemens Advia

Centaur XPT autoanalyzer. Vitamin D, T 3, T4 and TSH are assayed by the following methods:

1. VITAMIN D:Chemiluminescence Competitive immunoassay.
2. T 3 : Chemiluminescence Competitive immunoassay
3. T4 : Chemiluminescence Competitive immunoassay
4. TSH: Chemiluminescence Two -site sandwich immunoassay

Observation and results

The present study involved 150 subjects, out of which 50 are infertile female patient attending gynaecology outpatient are enrolled in the study in Gandhi hospital who fulfilled inclusion criteria and 100 were fertile females as controls. The present study was undertaken to evaluate serum vitamin D and Thyroid profile in infertility female patient. Serum vitamin D and Thyroid profile is estimated by chemiluminescence method by using Siemens Advia centaur xpt Autoanalyzer

Statistical analysis

The results were tabulated in master chart and statistically analysed using Microsoft excel software version 10. The descriptive results were expressed as mean and Standard deviation. Significance of difference between groups were assessed by applying Epi info software. The P- value were expressed along the mean values and standard deviation. Results on categorical measurements arepresented in Number and %. Significance is assessed at 5 % level of significance.

Significant figures

Significant (p value: $p < 0.05$) Highly significant (p value: $p < 0.01$) Not significant ($p > 0.05$)

Distribution of study sample according to age group

The distribution of the study samples according to the age were divided into 3 groups based on age18-24,25-31,32-39 years among cases and controls. There were 10 cases between 18-24 years,16 cases between 25-31 years and 24 cases between 32-39 years. There are 30 controls between 18-24 years, 34 controls between 25-31 years, and 36 controls between 32-39 years, which is showed in the table no.3.

Table 1: Sample distribution according to age group N=150

Age Group	Number ofCases	% of Cases	Number ofControls	% of Controls	Total
18 – 24	10	20%	30	30%	40
25 – 31	16	32%	34	34%	50
32 – 39	24	48%	36	36%	60
Total	**50**	**100**	**100**	**100**	**150**

The mean age of cases was 30.3 years and mean age of the controls was 28.6 years with Standard Deviation (SD) 6.3 and 6.3 respectively. The p-valueobtained on comparing the mean age of cases and controls was not significant (p- value > 0.05)

Table 2: Comparison of Mean ± SD of age between the two groups, N=150

Group	Mean Age	SD	p-Value
Cases (X = 50)	30.3	6.3	>0.05
Controls (Y=100)	28.6	6.3	

Table 3: Distribution of Serum vitamin D Levels in Cases and Controls. N=150

Vitamin D	Cases		Controls	
	No.	%	No.	%
<20 Deficient	37	74	41	41
20-29 Insufficient	10	20	49	49
>30 sufficient	3	6	10	10
Total	50	100	100	100

Table 4: Distribution of vitamin D deficiency based on severity among casesand controls

Vitamin DDeficiency	Cases		Controls	
	No.	%	No.	%
10-20 Mild	10	20	33	33
5 -10 Moderate	16	32	6	6
<5 Severe	11	22	2	2
Total	50	100	100	100

Table 5: Comparison of mean and standard deviation of serum vitamin Dbetween cases and controls

Group	Mean Ageof Vit. D	SD ofVit. D	p-Value
Cases (X = 50)	13.5	7.69	
Controls (Y=100)	20.5	6.43	<0.01

Table 6: Comparison of mean and standard deviation of T3, T4, TSH betweencases and controls

Parameters	Cases		Controls		p-value
	Mean	SD	Mean	SD	
T3	0.74	0.53	0.95	0.24	<0.01
T4	5.0	3.56	6.43	2.04	<0.05
TSH	6.40	4.02	3.68	1.02	<0.01

Table 7: Distribution of hypothyroid, hyperthyroid and euthyroid patients ininfertility cases

Thyroid profile	Cases	
Euthyroid	21	42%
Hyperthyroidism	4	8%
Hypothyroidism	25	50%
Total Cases	50	100%

Table-8: Distribution of vitamin D deficient among hypothyroidism cases ofinfertility

Thyroid Status	No. of Cases	No. of Vitamin D Deficient Cases	Distribution Vitamin D Deficiency According to Thyroid Profile (%)
Total No. of HypothyroidCases	24	22	91.6%
Total No. of EuthyroidCases	21	14	66.6%
Total No. of HyperthyroidCases	4	0	0%

Discussion

Infertility is a growing health issue around the globe, estimating 15% of population suffering from it. infertility causes may range from poor reproductive health of either partner or deficiencies of crucial biochemical substances like Hormones and vitamins, which are usually overlooked or undiagnosed[12]. It is the most prevalent chronic health disorder with worldwide prevalence of infertility around 60-80 million[13]. Endocrine system is the controller of organ functions in human body where hormones are messengers in endocrine system[14]. Majority of female infertility comprises of endometriosis, tubal damage and ovulatory dysfunction[15].

The effects of vitamin D are mediated by the vitamin D receptor, is a member of steroid receptor family which is expressed not only in calcium regulating tissues but also in reproductive organs such as ovary, uterus, placenta, testes, hypothalamus andpituitary. this diverse expression of VDR suggest role of vitamin D in reproductive physiology[16].

In ovaries, granulosa cell there is expression of VDR mRNA which has a role in steroidogenesis of sex hormone, their is a functional vitamin D response element VDRE in the promoter region of human AMH gene indicate effect of vitamin D on AMH, regulates HOXA10 expression in human endometrial stroma cells for the development of the uterus , for endometrial development, allowing implantation[17].

Normal hormone levels with sufficient endocrine activity is essential for successful implantation and maintenance of pregnancy. Thyroid dysfunction affects many organs including male and female gonads, interferes with human reproductive physiology, reduces the chances of pregnancy and adversely affects pregnancy outcome[13]. Hypothalamus-pituitary-thyroid and hypothalamus-pituitary- gonadal axis are interlinked, thus hypo and hyperthyroidism profoundly affect women fertility and altered thyroid physiology for long period leading to infertility[14]. Thyroid hormonesare essential for normal growth, sexual development and reproductive function. Hypothyroidism and hyperthyroidism are associated with delayed onset of puberty, menstrual disorders, anovulatory cycles, infertility[18]. Awareness of thyroid status in the infertile couple is crucial because of its significant, frequent and preventable effect on infertility[15].

Ovarian factors, tubal and peritoneal factors, anomalies, advanced ages above 35 years, hormonal disorders, habits, genetic factors, medical conditions and life style are the common risk factors for infertility. Identifying them and treating the cause can increases the chances of fertility[19]. With this background that the current study has been undertaken to assess the levels of serum vitamin D and serum thyroid hormones in infertile women and fertile controls.

Maheshwar et al[20] (2008) studied the effect of age on the diagnostic categories of infertility, data is collected from all couples attending Aberdeen fertility centre from 1993-2006.Total of 7172 women were studied, 26.9% over the age of 35 years. The mean age of infertile women was 31.2±5.2 SD. They found an association between female age and cause of infertility. Women over 35years of age are nearly twice as likely to present with unexplained Infertility. In the present study it was found that 48% of infertility cases belong to age group 32-39 years with mean±SD of age in infertility cases was 30.06±6.34 which is correlating with above study.

In weeg N et al[21] (2012) study focus on age related decline of infertility, which is correlating with the present study. Tulandi et al[22](1981) Pregnancy rate (33%) is much lower and the abortion rate (31%) is much higher with advancing age as compared with the general infertile population matching with the present study.

Garbedian et al[23](2013) found the effect of vitamin D status on

clinical pregnancy rates following invitro fertilization by cohort study on women undergoing IVF at Mount sinai Hospital. The prevalence of vitamin D deficiency, Insufficiency and sufficiency was 1.2%, 53.8%, 45.1%, found higher clinical pregnancy rate per IVF cycle started among women with sufficient vitamin D levels (52.5%) with mean ±SD 95.5±17.8 nmol/L than among women with insufficient levels (34.7%) with mean ±SD 54.5±14.0 nmol/L p value <0.001. In the present study distribution of vitamin D deficiency was mild 20%, moderate 32% and severe 22% in infertile cases, whereas distribution of vitamin D deficiency in fertile females was mild 33%, moderate 6%, severe 2% indicating majority of moderate and severe vitamin D deficiency belongs to cases with p value is highly significant <0.01 which is correlating with the above study.

Polycystic ovarian syndrome which is one of the most common endocrine disorder affecting women of reproductive age and is characterized by hyperandrogenism, menstrual disturbance and polycystic ovaries on ultrasound.

Dehghani Firouzabadi Raziah et al[24](2012) taken two groups I and II with mean age were 28.46 4.16 and 27.96 4.07. The mean levels of serum 25-OH-VD in groups I and II were 13.55± 6.39 and 13.21± 6.6 respectively. Group II received vitamin D supplementation, the level of serum 25-OH-VD increased from 13.21± 6.63 to 24.82± 6.54 in group II with significant p value< 0.001. In the present study distribution of vitamin D deficiency was mild 20%, moderate 32% and severe 22% in infertile cases with mean age 30.3±6.3 with vitamin D mean ±SD of cases was 13.5±7.69 with highly significant p value <0.01 correlate with the above study.

Vitamin D levels are influenced by various conditions such as Latitude and season, skin pigmentation, inadequate exposure to sunrays due to purdah system or use sunscreen or ageing. People living in countries at higher latitudes, such as the United States and Canada, are more prone to vitamin D insufficiency, especially during the winter months. A Canadian study by Veith R et al[25] (2001) reported prevalence of low 25(OH)D <40 nmol/l was higher in non-white, non-black subjects (25.6% of 82 women) than in the white women (14.8% of 702 white women, *P*<0.05). Of the 435 women tested during the winter half of the year November–April, prevalence of low 25(OH)D was not affected by vitamin D intake. In the present study vitamin D mean ±SD of cases was 13.5±7.69 and vitamin D mean ±SD of controls was 20.5±6.43 with significant p value <0.05 showing vitamin D deficiency is common in infertility cases may be due to various causes of vitamin D deficiency such as Latitude and season,inadequate exposure to sunrays due to purdah system or use sunscreen or ageing.other studies like Pagliardini L et al[26] and Shahrokhi SZ et al[27] shows role of vitamin D in reproduction and association of vitamin D deficiency with infertility

Ozkanet al[28] (2010) in a study on a group of 84 patients found positive correlation between the level of vitamin D in serum and follicular fluid and tendency to achieve clinical pregnancy (CP) following IVF (increased likelihood of achieving CP by 6%, p=0.030).In the present study distribution of vitamin D deficiency is more prevalent in infertile cases 74% compare to 41% of controls suggest vitamin D role inreproduction.

LataI et al[29] (2017) studied vitamin D levels in infertile females and correlation of vitamin D deficiency with AMH levels in comparison to fertile females .In that study vitamin D deficiency was present in 64.28% of infertile females with vitamin D deficient cases mean was 6.18±2.09 and AMH was 1.94±1.30 whereas mean of controls for vitamin D 4.85±3.02 and AMH was 3.47±2.59.On comparison vitamin D levels were lower in fertile than infertile females with p value 0.04 and AMH levels were lower in cases than control p value 0.003 is due to different study group taken in Lucknow, Uttar Pradesh. In the present study majority of cases had vitamin D deficiency 74% compare to 41% of controls. Vitamin D mean ±SD of cases was 13.5±7.69 with highly significant p value <0.01 showing vitamin D deficiency iscommon in infertility cases.

Nasir S et al[13](2016) s cross-sectional study of role of thyroid dysfunction in infertile women with menstrual disturbances, studied 168 infertile patients at Peshawar Medical College, from 21st April 2013 to 10th August 2013. Estimation of serum levels of thyroid hormones (TSH, T3 and T4) were performed on Chemiluminescence Immunoassay Analyzer. Among 168 infertile women 106 (63.09%) primary infertility and 62 (36.91%) secondary infertility. The mean age of primary infertile women was 26.74±4.82 years and secondary infertile women 30.52±4.74 years. Distribution of hypothyroidism 3(1.78%), hyperthyroidism 31(18.45%), euthyroid 134(79.76%), showing hyperthyroidism is more commonly associated with menstrual abnormalities in infertility with significant p value of 0.01 indicate association of menstrual irregularities with abnormal thyroid profile in infertility. In the present study it was found that 48% of infertility cases belong to agegroup 32-39 years with mean± SD of age in infertility cases was 30.06±6.34 .

In the present study thyroid hormones (TSH, T3 and T4) were analysed by Advia centaur xpt Siemens based on Chemiluminescence Immunoassay method. Distribution of hypothyroidism 25(50%), hyperthyroidism 4(8%), euthyroid 21(42%), showing hypothyroidism is the most common altered thyroid status observed in infertility with mean ± SD of T3 0.74±0.53 ,mean ± SD T4 is 5.06±3.56 in cases, mean ± SD of TSH is 6.40±4.02 in cases with highly significant p value <0.01 which shows thyroid disorders are one of the important cause of infertility correlating with the above study.

Nath et al[30](2015) found the mean ± SD of TSH, FT3, FT4, and PRL were4.22 +/- 3.42 mIU/mL, 4.02 ± 2.3, 1.13 ± 1.21, and 14.33 ± 10.71, respectively, in infertile patients. Out of 53 infertile patients, 24% [13] were found to present hypothyroidism and only 3% [2] hyperthyroid. When t-test was done, statistically significant higher prolactin (p = 0.001) and TSH (p = 0.001026) levels were found among infertility cases. In present study mean ± SD of T3 0.74±0.53 in cases, mean ± SD T4 is 5.06±3.56 in cases, mean ± SD of TSH is 6.40±4.02 in cases with highly significant p value<0.01 and distribution of hypothyroidism constitute about 50% of infertility cases, hyperthyroidism was 8% and euthyroid was 42% correlating with the above study.

Surekha T Nemade et al[15](2012) was a case control study carried out in central clinical laboratory, in Dr. Vasant rao Pawar Medical College, Hospital and research centre, Nashik. The study included 50 females between age group 20-40 years, diagnosed as primary infertility and compared with 50 age matched fertile females, Serum T3, T4 and TSH levels were determined by immunoenzymaticmethod using Acculite CLIA microwells manufactured by LUMAX. T3, T4 and TSH levels in control (fertile) and primary infertility females T3 (ng/ml), T4 µg/dl, TSH µIU/ml Fertile females mean ± SD 0.89 ± 0.35, 6.90 ± 1.97, 4.81 ± 2.14 whereas mean ± SD Primary infertile females is 1.4 ± 0.56, 8.60 ± 3.04, 3.46 ± 1.85 with p< 0.01- highly significant. But in present study mean ± SD of T3 0.74±0.53 in cases, mean ± SD T4 is 5.06±3.56 in cases, mean ± SD of TSH is 6.40±4.02 in cases with highly significant p value<0.01.Thus altered thyroid profile can result in infertility indicated by above studies.

The pleiotropic roles of vitamin D have been recognized through various studies which suggested a beneficial role of vitamin D in the management of thyroid disease. Recent evidence has demonstrated an association between low vitamin D status and autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves'disease[31].

Mansournia N et al[32] (2014) found Serum 25OHD and TSH levels (on the log scales) were negatively correlated among the case group (P = 0.03), but not among the control group (P = 0.61).In the present study vitamin D deficiency is commonly seen in hypothyroid patients, negative correlation between TSH and vitamin D is observed with highly significant p value<0.01.

Limitations

The limitations of the present study were, that the study was conducted on a limited sample over a short duration of time. Large scale study over long period is required to observe the more significant changes.

Scope for Future Study

- Further study is needed with more research at molecular level,

to find out the causes and predisposing factors leading to infertility.

- Improved analytical method is needed which help in better diagnosis andscreening of infertility.
- Multiple parameter should be taken with higher sensitivity and specificity helpin better diagnosis and prevention.

Conclusion

Infertility can cause societal repercussions, personal suffering, psychological effects, clinical depression, sexual dysfunction and stress. In the society childlessness is a challenging condition to the couple and has to face social and family problems. Hence proper investigations with hormonal assessment and supplementation is needed for the management of infertility.

This study shows negative correlation between vitamin D and TSH levels which need to be further evaluated. The results of our study support the hypothesis that vitamin D if received properly through sunlight and diet can decrease the chances of infertility and proper early management of thyroid disorders is useful for better infertility treatment. Hence all patients of female infertility should be screened for vitamin D and thyroid profile assessment.

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