Research Article

Association of Free Testosterone and DHEA-S with Dyslipidemia in Women with Polycystic Ovarian Syndrome- a case-control study

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Keywords

androgen, DHEA-S, dyslipidemia, free testosterone, infertility, insulin resistance, PCOS

Abstract

Background: Polycystic ovarian syndrome (PCOS) is a multisystem disorder presenting with menstrual irregularities, infertility, and features of hyperandrogenism. Hyperandrogenism predisposes to the critical clinical features of PCOS. This find aimed to study the association of androgenic hormones such as dehydroepiandrosterone sulfate (DHEA-S) and free testosterone with lipid profile in PCOS women.

Methods: This case-control study was conducted in the Department of Biochemistry at a tertiary care hospital in Chennai. Patients were recruited from the Department of Obstetrics and Gynecology. Participants were aged 18-40 years. Blood samples were collected for analysis of lipid profile, DHEA-S, and free testosterone. DHEA-S and free testosterone were analyzed by ELISA. Ethics approval and written informed consent were obtained. Based on the distribution of the data, appropriate statistical tools were used. P-value ≤ 0.05 was considered statistically significant.

Results: Most of the participants were aged between 21 and 30 years. HDL-c was decreased in PCOS patients compared to healthy individuals; however, no statistically significant difference was found. Free testosterone showed an association with triglyceride. The areas under the curves of DHEA-S and free testosterone were 0.638 and 0.765, respectively.

Conclusion: DHEA-S and free testosterone showed good area under the curves. But free testosterone performed better with a higher area under the curve as well as its association with triglyceride. The cut-off values to diagnose PCOS were 3.0 µg/mL and 2.5 pg/mL for DHEA-S and free testosterone, respectively, with adequate sensitivity and specificity. Since free testosterone performed better in ROC curve than DHEA-S, free testosterone is considered to be a potential biomarker of identifying hyperandrogenism in PCOS women.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age group [1]. PCOS presents with a worldwide prevalence rate from 2.2% to 22.5% [2]. Whereas the prevalence in India is 28.9% as reported by the National Institute of Health (NIH) criteria, 35.3% by the Rotterdam criteria [3], and 34.3% by the Androgen Excess–PCOS Society (AE-PCOS) criteria [4]. PCOS is caused by genetic, epigenetic and nongenetic causes. In this metabolic condition, hyperinsulinemia with insulin resistance (IR) is highly prevalent among these women. It predisposes to the development of type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), and cardiovascular disorders (CVD) [5,6]. Generally, the ovaries and the adrenal glands are involved in the synthesis of steroid hormones. Around 90% of dehydroepiandrosterone (DHEA) and its sulphate ester (DHEA-S) are mainly produced in the adrenal glands. Approximately 40-70% of PCOS women have increased levels of androstenedione and DHEA-S. Studies have shown contradictory evidence between IR and DHEA-S levels. DHEA is found to positively influence the antral follicle count and ovarian volume of women undergoing assisted reproduction for primary ovarian insufficiency [7].

Studies indicate that high androgen levels play crucial roles in the pathogenesis of PCOS. High testosterone levels are linked with abdominal fat distribution, IR and glucose intolerance in obese individuals [8]. PCOS women with elevated free testosterone have an adverse metabolic profile compared to PCOS women with normal androgen levels [6]. IR is associated with dyslipidemia as evidenced by decreased levels of high-density lipoprotein cholesterol (HDL-c), increased levels of low-density lipoprotein cholesterol (LDL-c) and triglyceride (TGL) [9]. Various androgen markers like androstenedione, sex hormone binding globulin (SHBG), DHEA-S, free testosterone, and total testosterone have been analyzed to diagnose hyperandrogenism [10].

The metabolic sequelae in women with PCOS could be due to the activation of the renin–angiotensin–aldosterone system (RAAS), a dysfunctional autonomic nervous system, and increased synthesis and activity of androgen receptors [11]. To screen women with suspected hyperandrogenism, levels of DHEA-S and free testosterone are being analyzed. This study aimed to find the association of free testosterone and DHEA-S with dyslipidemia in women with PCOS.

Methods

The case-control study was conducted in the Department of Biochemistry at a tertiary care hospital in South India.

Study participants

The study included 180 women of reproductive age from 18 to 40 years; healthy women did not have the features of PCOS (n=90). Individuals who presented with PCOS (n=90) with diagnosis based on the Rotterdam criteria were treated as cases. Individuals with diabetes mellitus, endocrine, liver, and renal disorders, and women on oral contraceptive pills, anabolic or androgenic drugs, smokers, and alcoholics were excluded from the study. The study participants were recruited from the Department of Obstetrics and Gynecology.

Ethics statement

Ethics approval was obtained from the Institutional Ethics Committee (Ethics No.: CSP-MED/18/AUG/45/109, dated 24-09-2018). The participants provided written informed consent before participating in the study.

Sample collection

Blood samples from the study participants were collected in red-topped vacutainers by trained phlebotomists. These samples were centrifuged at 3200 rpm for 15 minutes. The separated serum was analysed for free testosterone, DHEA-S, and lipid profile.

Analysis of the parameters

Free testosterone and DHEA-S were assayed by using a competitive ELISA from Diametra, Italy. This assay employs a competitive assay format based on the ELISA principle first described by Engvall and Perlmann (1971) and refined in standard immunoassay protocols for small steroid hormones [12]. Lipid profile was analyzed in the hospital central laboratory by standard methods.

Funding statement

The study received financial support from the Indian Council of Medical Research through the ICMR-TSS (Talent Search Scheme) fellowship grant with registration no: U04M170045.

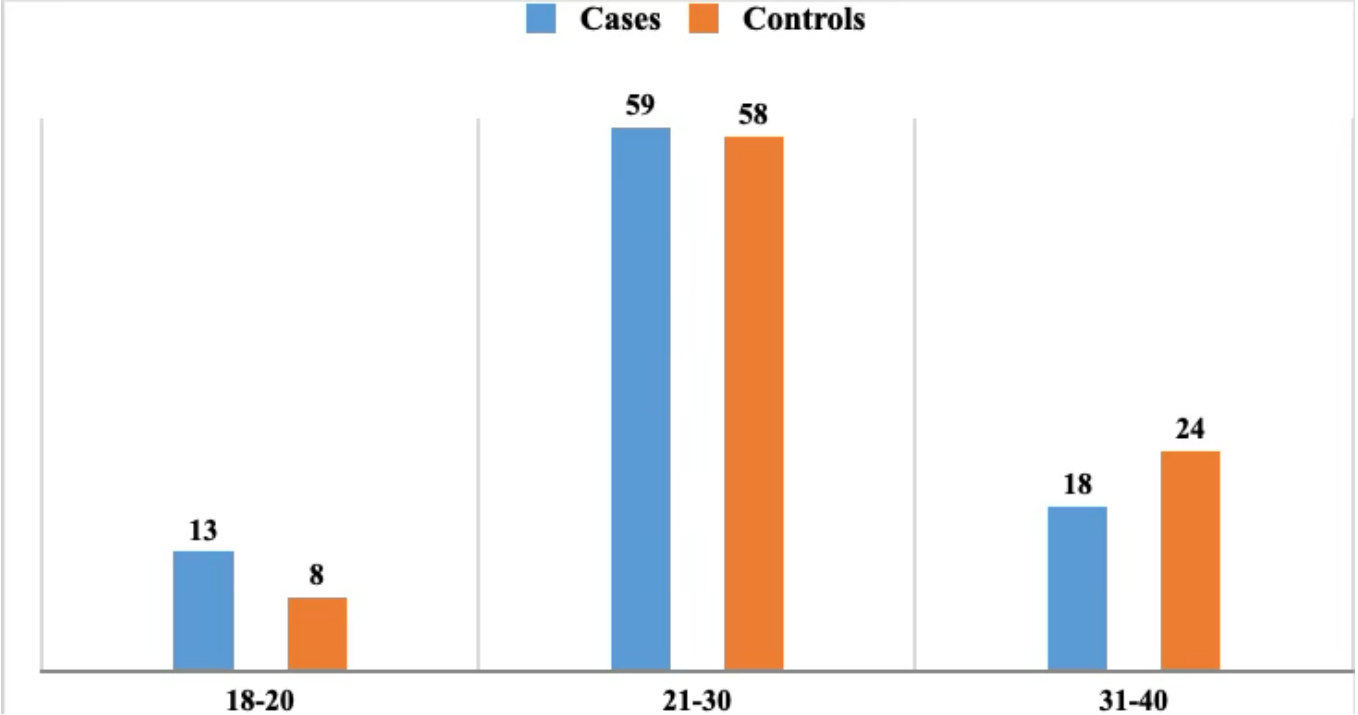
Statistical analysis

The obtained data were subjected to the Kolmogorov-Smirnov test. Based on the type of distribution, mean and standard deviation, or median and interquartile range (IQR) were used. To compare the variables between the groups, Student's t-test or the Mann-Whitney U test was used for continuous variables and the Chi-square test was used for categorical variables. Spearman's Correlation was done to find the association among the variables. A Receiver Operating Characteristics (ROC) curve was analysed and Youden index was used to arrive the cut-off values of DHEA-S and free testosterone. Statistical analyses were performed with SPSS version 16.0. A p-value $\leq$ 0.05 was considered statistically significant.

Results

Figure 1 shows the distribution of the participants according to the age. Among 90 PCOS individuals, 13 belonged to the age 18 - 20 years, 59 belonged to the age 21 - 30 years, and 18 belonged to 31 - 40 years. Among controls, eight belong to 18 - 20 years, 58 belonged to 21 - 30 years, and 24 belonged to 31 - 40 years (Figure 1).

Figure 1: Age-wise distribution of study participants.



Baseline characteristics

The data in Table 1 were compared between PCOS cases and controls given in columns, 3 and 4 respectively. The mean age of the individuals in the case and control groups was 26.77 and 27.56 years, respectively, with no statistically significant difference in age between the groups (p=0.40). There was no statistically significant difference in age distribution among the groups (p=0.35) (Table 1).

Table 1: Comparison of biochemical variables between PCOS women and controls.

Characteristic	Overall (n = 180)	PCOS (n=90)	Control (n = 90)	p-value
Age (year)	27.16 (5.80)	26.77 (5.37)	27.56 (6.21)	0.4
18-20 years n (%)	21 (11.7)	13 (14.4)	8 (8.9)	0.35
21-30 years n (%)	117 (65)	59 (65.6)	58 (64.4)	
31-40 years n (%)	42 (23.3)	18 (20)	24 (26.7)	
DHEA-S, µg/mL	2.3 (1.2-5.0)	2.7 (1.6-5.8)	1.9 (1.0-3.5)	<0.001**
Free testosterone, pg/mL	1.95 (1.0-2.95)	2.3 (1.3-4.2)	1.2 (0.9-2.0)	< 0.001**
Total cholesterol, mg/dL	135.53 (25.30)	134.52 (23.99)	136.53 (26.63)	0.6
TGL, mg/dL	98.84 (33.01)	100.72 (36.79)	96.96 (28.83)	0.4
HDL-c, mg/dL	39.61 (3.75)	39.13 (3.39)	40.09 (4.04)	0.08
LDL-c, mg/dL	76.17 (22.76)	75.21 (21.67)	77.13 (23.88)	0.6
Normal lipids n (%)	65 (36.2)	29 (32.2)	36 (40)	0.27
Dyslipidemia n (%)	115 (63.8)	61 (67.8)	54 (60)	

Total cholesterol, TGL, HDL-c, LDL-c expressed in mean and standard deviation, compared by student's t-test; DHEA-S, Free testosterone: expressed in median and interquartile range, compared using Mann-Whitney U test; Age, normal lipids, dyslipidemia: expressed in frequency and percentage, compared using Chi-Square test; * p-value: statistically significant; **p-value: Highly statistically significant

In the present study, the median values of DHEA-S were 2.7 in PCOS and 1.9 µg/mL in controls, and there was a statistical difference (p<0.001) between the groups. The mean levels of free testosterone were 2.3 in cases and 1.2 pg/mL in controls. There was a high statistical difference between the groups (p<0.001) (Table 1). There was no statistically significant difference between the groups for total cholesterol (p=0.6), TGL (p=0.4), HDL-c (p=0.08), and LDL-c (p=0.6) (Table 1).

Alterations of lipid profile

The lipid profile data were characterized by National Cholesterol Education Program (NCEP)-Adult Treatment Panel (ATP)-III guidelines [13]. Total cholesterol, TGL, and LDL-c were within the acceptable limits in both groups. However, HDL-c was normal in the control group but lower in PCOS. Around 32.2% of individuals in cases and 40% of individuals had normal lipids; whereas, 67.8% of individuals in PCOS and 60% of individuals in controls had dyslipidemia, which was statistically not significant (p=0.27) (Table 1).

Spearman correlation was done for DHEA-S and free testosterone with other parameters, as shown in Tables 2 and 3, respectively.

Table 2: Spearman correlation of DHEA-S with the other parameters.

Parameter	Correlation coefficient ('ρ' value)	p-value
Free Testosterone	0.115	0.12
Total cholesterol	0.007	0.92
TGL	-0.061	0.41
HDL-c	0.06	0.42
LDL-c	0.016	0.82

* p-value: statistically significant; ** p-value: Highly statistically significant

Spearman correlation of DHEA-S with other parameters did not show any statistical difference (Table 2).

Table 3: Spearman correlation of free testosterone with other parameters.

Parameter	Correlation coefficient ('ρ' value)	p-value
Total cholesterol	-0.027	0.71
TGL	0.212	0.004**
HDL-c	-0.123	0.1
LDL-c	-0.067	0.37

* p-value: statistically significant; ** p-value: Highly statistically significant

Table 4: Area under the curve and cut-off values of DHEA-S and free testosterone.

Biomarker	AUC	95% CI	p-value	Sensitivity (%)	Specificity (%)	Optimal Cut-off
DHEA-S	0.765	0.551 – 0.718	<0.001**	59	67	3 µg/mL
Free testosterone	0.765	0.692 – 0.832	<0.001**	68	81	2.5 pg/mL

Free testosterone showed larger AUC compared to DHEA-S. Sensitivity and specificity are larger for free testosterone compared to DHEA-S (Table 4).

Discussion

In the present study, around 65% of the participants in both cases and controls were in 21- 30 years age group. This was further emphasised by the mean age of around 27 years in both groups. DHEA-S and free testosterone were higher in PCOS compared to that of controls. There was no significant difference in lipid profile between cases and controls. All the lipid profile parameters were within the biological reference intervals except HDL-c which was lower in cases. Free testosterone showed correlation with TGL as well as better performance in the ROC curve.

PCOS is a common metabolic disorder prevalent among reproductiv age group women. It is characterized by ovarian dysfunction, hyperandrogenism, and polycystic ovarian morphology. Patients present with menstrual irregularities, infertility, hirsutism, acne, obesity, and features of MetS. Also, hyperandrogenism in PCOS has significant long-term implications, such as increased risks of T2DM, CVD,

endometrial hyperplasia, and psychosocial impacts. Hence early diagnosis and treatment is mandatory.

The study participants were aged between 18 and 40 years, 65% were aged 21 to 30 years in both groups. The mean ages of the individuals in the PCOS and control groups were 26.77 and 27.56 years, respectively (Table 1). The present study findings about age of the participants were in alignment with Vijayan et al 2022. PCOS is prevalent in women of approximately 26 years, and these individuals have higher Anti-Mullerian Hormone (AMH) compared to healthy controls [14]. Gupta et al (2018), found a PCOS prevalence of 8.2% among college girls aged 17 to 24 years [15]. The prevalence (22.5%) is higher in a study conducted in Mumbai, among 600 girls aged 15–24 years [16]. Thus, prevalence varies according to the study population and the criteria used to diagnose PCOS.

PCOS is associated with increased risk of IR, obesity, dyslipidaemia, and MetS. Studies show that among the lipid profile variables serum levels of total cholesterol, LDL-c, and TGL get elevated, while the level of HDL-c gets lowered in PCOS women compared to that of controls [17]. As per Mehreen et al study, symptoms of hyperandrogenism, hyperinsulinemia, and adiposity are commonly seen in PCOS. This is associated with weight gain and male pattern distribution of body fat. Among South Indian adolescents with PCOS, weight gain is common among women with menstrual disorders. The male pattern of body fat distribution in PCOS is due to hyperandrogenism. This facilitates a vicious circle of hyperinsulinemia, hyperandrogenemia, central obesity, and metabolic dysfunction [18]. Hyperandrogenism also influences unfavorable dyslipidemia in these individuals. In addition to hyperandrogenism, other factors such as IR, environmental factors, and genetics play roles in the setting and progression of dyslipidemia. Obesity as measured by anthropometric measurements such as body mass index (BMI) and waist circumference (WC) shows good correlation with dyslipidemia, especially in South Indian PCOS women [19].

According to Parveen et al, BMI, WC, and waist-to-hip ratio (WHR) are elevated in PCOS. Among lipid profile, total cholesterol showed a positive correlation with TGL among PCOS patients [20]. Begum et al, in their study found that 58% of the participants are overweight while 15% are obese, and 25% have normal BMI. In addition, PCOS individuals have higher fasting plasma glucose levels compared to controls [21]. PCOS women with large WC have lower HDL-c levels, apolipoprotein A1 (Apo A1), and albumin levels compared to controls. In PCOS, testosterone levels are associated with higher very low-density lipoprotein (VLDL) and elevated WC compared to controls. The higher testosterone levels, are also associated adversely with insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR) in PCOS cases compared to controls [22].

In this study, there was no statistically significant difference between the groups among lipid profile- total cholesterol ($p=0.6$), TGL ($p=0.4$), HDL-c ($p=0.08$), and LDL-c ($p=0.6$) based on NCEP-ATP III guidelines. Total cholesterol, TGL, and LDL-c were within acceptable limits in both groups. However, HDL-c was below the cut-off level in controls but lower in PCOS. Around 67.8% of PCOS and 60% of controls had dyslipidemia (Table 1). According to Conwell et al, IR is at the crossroads in the pathogenesis of dyslipidemia, glucose intolerance, and hyperandrogenism irrespective of BMI. The surrogate markers of IR include the HOMA-IR, the quantitative insulin sensitivity check index (QUICKI), and the fasting glucose to insulin ratio (FG-IR) [23]. TGL-to-HDL-c ratio of 3.5 predicts the presence of the small dense LDL phenotype, which is considered to be the most atherogenic lipoprotein. This ratio is better than the total cholesterol-to-HDL-c ratio, HDL-c alone, or TGL alone. Thus, it gives an insight into lipoprotein metabolism that increases CVD risk in insulin-resistant patients [24]. According to Macut et al, lipid levels are almost the same in both PCOS women and controls. But PCOS women show characteristic features of IR, such as decreased HDL-c and increased TGL. Impaired clearance of small dense LDL-c and the association of free fatty acids and apolipoprotein E (Apo E) to IR, indicate worsening of metabolic status and premature onset of atherosclerosis [9].

With the onset of IR and hyperinsulinemia, hyperandrogenaemia facilitates the formation of visceral adipose tissue, which exacerbates the secretion of androgen in the ovaries and adrenal glands [25]. The excess androgen hormones produced by the ovaries and adrenal glands are converted to DHEA-S and testosterone. This leads to increased levels of DHEA-S and testosterone in PCOS [26]. Though testosterone circulates in plasma as both free or bound form (bound to SHBG and albumin), free testosterone is found to be a sensitive marker in the diagnosis of PCOS [10]. A study conducted in Belgium showed that 44% of PCOS individuals have high free testosterone levels [10]. In the present study, the median levels of DHEA-S and free testosterone were higher in cases than in controls, and were statistically significant ($p<0.001$) (Table 1). Spearman correlation of DHEA-S with other parameters did not show any statistical difference (Table 2); but free testosterone showed a high statistically significant correlation with TGL ($p=0.004$) (Table 3).

The findings of the study by Christodoulaki et al. revealed that there is a negative correlation between DHEA-S levels and the ovarian volume. The precise pathophysiology behind this association remains unknown. However, this effect might be linked to increased fibrosis of ovarian tissue, which ultimately leads to menstrual disorders and decreased fertility potential [7]. About half of the testosterone is produced by the peripheral conversion of adrenal androgens, and elevated DHEA-S levels also contribute. The DHEA-S/free testosterone ratio might be a more accurate measure of the metabolic effects of androgens. An extra-adrenal mechanism likely to be involved in regulating adrenal androgen release; other than the influence of adrenocorticotropic hormone (ACTH). In PCOS, central adiposity is negatively correlated with serum DHEA-S levels.

PCOS metabolic tests are correlated with higher levels of free testosterone. Thus, DHEA-S/free testosterone ratio is better than the individual hormones to monitor metabolic parameters in PCOS [26].

In the present study, DHEA-S showed an AUC of 0.638 and the cut-off of 3.0 µg/mL with sensitivity and specificity of 59% and 67% respectively. Free testosterone showed a higher AUC of 0.765 with cut-off of 2.5 pg/mL with sensitivity and specificity of 68% and 81% respectively. Since free testosterone showed higher AUC compared to DHEA-S, free testosterone could be a better indicator of hyperandrogenism. (Table 4) Adrenal hyperandrogenism is common in patients with non-classic (B and C) phenotypes of PCOS and is due to higher production of androgens in PCOS patients. However, other factors may increase the adrenal androgen production and influence the clinical expression of the syndrome [27]. In a German PCOS cohort, ROC curve analysis suggests that calculated androgen indices (bioavailable testosterone, free androgen index, free testosterone) are superior to other androgen variables in defining PCOS and represent reliable markers for androgen excess [28].

To investigate the function of free testosterone and androstenedione in the metabolic phenotype of PCOS, Lerchbaum et al. undertook a study in Austria, which showed that PCOS women who had elevated free testosterone with normal androstenedione levels are at a higher risk for metabolic problems [6]. Elevated free testosterone levels are observed in approximately 70% of PCOS patients, especially those diagnosed by the NIH 1990 criteria. The present recommendation is to measure free testosterone concentration and SHBG. Approximately 20% to 30% of patients with PCOS have only high levels of DHEA-S; circulating levels of DHEA-S have limited diagnostic value. Hence, serum androgens, including free testosterone, should be used as an adjuvant tool for the diagnosis of hyperandrogenic disorders [8].

Patients with PCOS should also closely monitor their lipid profiles for the presence of elevated apolipoprotein B100 (Apo B100). A 2.8-fold causative increase in the risk of ischemic heart disease is associated with a non-fasting increase of 1 mmol/L in Apo B lipoprotein remnants, which is correlated with fasting insulin and IR. Dietary control and lifestyle changes are the mainstays for the treatment of dyslipidemia. Additionally, targeted lipid-lowering medications may normalize dyslipidemia; however, this is not yet a recognized treatment for PCOS. The optimal subset of PCOS women who need lipid-lowering therapy and the efficacy of such treatments for dyslipidemic women will need to be determined by future clinical research [29].

Limitations

The PCOS individuals were not classified according to the various phenotypes of PCOS. The lipids and androgens were not compared with insulin resistance. Anthropometric measurements and ovarian morphology were not included in the study.

Conclusion

Most of the study participants were in the third decade. Among the lipid profile markers, HDL-c was lesser than cut-off level among the PCOS individuals. The free testosterone was positively correlated with triglyceride, whereas DHEA-S did not show any association with lipid profile. The ROC curve analysis showed larger area under the curve for free testosterone compared to DHEA-S; thus, indicating that free testosterone could be a sensitive marker for diagnosing PCOS. However, further studies with large sample size is required.

Declarations

Ethics statement

All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethics approval was obtained from the Institutional Ethics Committee (Ethics No.: CSP-MED/18/AUG/45/109, dated 24-09-2018). The participants gave written informed consent to participate in the study.

Funding statement

The study received financial support from the Indian Council of Medical Research through the ICMR-TSS (Talent Search Scheme) fellowship grant with registration no: U04M170045.

Conflict of interests

The authors wish to declare that there were no conflict of interests either during the research or while writing this manuscript.

Data availability

Data obtained from the study will be with the corresponding author for a period of five years and the same can be shared for those working in the same field. The data will be destroyed after five years of publication.

Authors' contributions

Conceptualization: Lavanya K, Palaniappan N, Santhi Silambanan, Methodology: Lavanya K, Palaniappan N, Santhi Silambanan, Vinodhini VM, Formal analysis: Lavanya K, Santhi Silambanan, Vinodhini VM, Mahesh Kumar, Investigation: Lavanya K, Palaniappan N, Santhi Silambanan, Resources: Lavanya K, Palaniappan N, Santhi Silambanan, Vinodhini VM, Data curation: Lavanya K, Palaniappan N, Santhi Silambanan, Mahesh Kumar, Writing- original draft: Lavanya K, Palaniappan N, Santhi Silambanan, Writing-Review & editing Supervision: Lavanya K, Palaniappan N, Santhi Silambanan, Vinodhini VM, Mahesh Kumar, Project

administration: Lavanya K, Palaniappan N, Santhi Silambanan, Vinodhini VM, Funding acquisition: Lavanya K, Santhi Silambanan. All the authors have contributed equally in writing the final manuscript.

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