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EVALUATION OF ASSOCIATION OF ANTHROPOMETRIC INDICES WITH STRESS RESPONSE IN PCOS POPULATION

Physiology

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ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is a complex endocrinopathy of women in their reproductive age with diversity in clinical manifestations. The phenotypic expressions and altered anthropometric indices are the key indicators of the abnormal transformations in metabolic, endocrine, and reproductive functionality of PCOS. Stress-induced activation of the Sympathetic-Adrenal-Medullary (SAM)-system and Hypothalamic-Pituitary-Adrenal (HPA)-axis results in series of neuroendocrine adaptations where over-activation of SAM/HPA response negatively affects the Hypothalamic-Pituitary-Gonadal (HPG) axis that highlight the possibility of the adverse impact of stress response system on reproductive and metabolic functions. Psychological distress and anxiety induced by the COVID-19 pandemic may aggravate the expressivity of PCOS and associated complexities. **Objective:** The present study aimed to find out some phenotypic features (hirsutism, acne, Acanthosis Nigricans (AN) and alopecia) in association with deviated anthropometric and derived indices, and stress biomarkers (Salivary α-amylase (SAA) and cortisol of SAM and HPA-axis respectively) among PCOS patients. **Statistical analysis:** SPSS (IBM, version 20) and Microsoft Office Excel 2010 were implied, and the quantitative variables were described as mean±SD ($P < 0.01$ and $P < 0.05$). **Results:** Significant alternations in the anthropometric indices were observed for PCOS patients that may lead to health risk. Alteration in body composition was strongly correlated with stress biomarkers in the case of both the experimental and control group. Central obesity was found to be a significant indicator of altered body composition in response to stress. **Conclusion:** Stress is a key modulator in the alternation of phenotypic manifestation as well as body composition of PCOS patients which could be the plausible reason for further deterioration of this multifaceted disorder due to the COVID-19 pandemic.

KEYWORDS

PCOS, stress, α-amylase, cortisol, COVID-19.

INTRODUCTION:

Polycystic Ovary Syndrome (PCOS) is a multifactorial complex disorder where multiple genetic variants, various biochemical parameters, and diverse environmental components involved and crosstalk in the expression of it [1]. A stress response is essential for sustained adjustments that promote survival but prolonged stress copes up it at a greater health cost. Scientific and clinical shreds of evidence suggest that chronic stress causes reproductive compromise along with neuroendocrine, metabolic, and behavioral responses [2]. Excessive activation of the stress axes-HPA-axis and SAM-system, and its reciprocal relation with the Hypothalamic-Pituitary-Ovarian (HPO) axis might be a leading cause of emerging trend of various reproductive dysfunction like PCOS [3], [4], the most prevalent factor for menstrual disturbances and infertility of female in their reproductive age [5], [6]. Stress-induced desynchronized pulsatile gonadotropin (GnRH) release from the hypothalamus (HT) alters the secretory pattern of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) from the pituitary, as well as HPA axis and results in clinical manifestations of the polycystic ovary (PCO), hyperandrogenism and insulin insensitivity [5]-[7]. Type-II diabetes, a metabolic consequence of PCOS is reflected by increasing amount of circulating insulin that binds with the receptor of Insulin-Like Growth Factor-I (IGF-I) and stimulates 5-α reductase (enzyme for the conversion of testosterone to dihydrotestosterone, DHT) activity resulting in cutaneous manifestation of hyperandrogenism like hirsutism (androgen-dependent male pattern hair distribution in female), acne (androgen-induced comedones formation), AN (hyper-pigmented skin) and alopecia (progressive thinning of hair) [8]. A large number of genetic variants related to insulin resistance(IR), regulation of androgen biosynthesis and function, metabolic syndrome (MetS), and pro-inflammatory genotypes are intertwined with each other and play an important role in the expression of PCOS [9]-[12]. In a recent report Kyrou I, et al. pointed out that the individuals having PCOS might be in a potential risk zone to catch COVID-19 as both the diseases share an overlapping metabolic and anthropometric [13] as well as the genetic platform.

Anthropometric indices are sensitive indicators of the health of a population and useful for understanding the variation of body composition. Alteration of parameters like central adiposity, Body-Mass-Index (BMI), Waist-Hip-Ratio (WHR), and muscle mass was found to be useful indicators for the prediction of various diseases [14]-[17]. Studies by different groups suggest that there are ethnicity-related differences in anthropometric profile and body composition in women suffering from reproductive disorders [18], [19]. Obesity and fat distribution pattern are supposed to play an important role in the aetiology of PCOS [14]-[17], [20], [21]. About 40% of women with PCOS are obese with abnormal abdominal fat distribution [21]. Our previous study supports the hypothesis that stress or more precisely stress-associated factors are positively associated with body composition alterations in PCOS individuals, as compared to age-matched controls [4]. Salivary α-Amylase (SAA) and cortisol are the major stress mediators [4], [22-24] but their role in reproductive disorders is yet to be established. In the present study we tried to evaluate the relation of some central obesity indicators like Waist-Circumference (WC), Waist-to-Stature-Ratio (WSR), or Waist-to-Height Ratio (WHtR), and Body-Adiposity-Index (BAI) with SAA and cortisol in PCOS patients. We initiated the present study by observing the phenotypes followed by the estimation of anthropometric indices to attempt the conclusion of crosstalk between central obesity and stress biomarkers. Ponderal Index (PI) is a measure of leanness (corpulence) of a person [25] and has been shown to have higher sensitivity and specificity, as well as both positive and negative predictive values than BMI [26]. Body-Surface-Area (BSA) is a good indicator of metabolic mass than often beneficial for understanding health hazards [27]. We also attempted to find out the relation of Body-Fat (BoF), Visceral-Fat Level (VFL), and Subcutaneous-Fat (SF) with their distribution in the Whole Body (SWBF), Trunk (STF), Arms (SAF), and Legs (SLF) as risk factors of PCOS [28]. WC, WSR, and BAI are useful parameters to quantify central obesity that is a key indicator of PCOS. Skeletal-Muscle (SM) is a potential indicator of hormonal disturbance associated with PCOS [17].

2.3 Assay of Stress Biomarkers

2.3.1 Collection of Saliva

The study subjects were allowed to sit upright in a comfortable position by tilting the head forward, for pooling saliva on the floor of the mouth to be passed through the Saliva Collection Aid (SCA-Salimetrics, Item No. 5016.02) in a polypropylene vial. Samples of saliva were collected by the passive drool using SCA in ice-chilled 1.8 ml cryovial and immediately frozen [29], [30].

2.3.1.1 Assay of SAA

Samples of saliva were centrifuged at 3000 rpm for 15 min and the clear supernatant was processed immediately for measurement of A-A. The assay employed 2-chloro-4-nitrophenol (chromogenic substrate), linked to maltotriose (CNP-Gal-G2). A-A catalyses the hydrolysis of CNP-Gal-G2 to CNP and the hydrolysis rate was measured as an enhancement in absorbance due to the formation of CNP that is proportional to the A-A activity in the sample (Direct Substrate Method, Kinetic Enzymatic, Crest Biosystem, Goa, India) [4].

2.3.1.2 Assay of salivary cortisol

Saliva-cortisol-ELISA assay kit from Diametra (using ELISA-reader-Biorad-680) was used to assay salivary cortisol. During analysis, the wells of the ELISA plates were marked as Calibrators, Samples, and Blank. Then 25 µL of samples and each calibrator were taken in the respective wells and 200 µL of the diluted conjugate was added to each well except the Blank. Then this was incubated at 37° C for 1 hr. After that, the contents of the wells were removed and washed with 300 µl of diluted wash solution 3 times. TMB substrate (100 µl) was added to all the wells (including the Blank) and incubated for 15 min at room temperature in the dark with gentle shaking. At the termination of incubation, stop solution (100 µl) was added and the absorbance was measured at 450 nm against blank within 5 min. The best fit curve was obtained by plotting the mean value of the absorbance of the calibrators against concentration. The values of absorbance of samples were interpolated on the calibration curve for obtaining the corresponding values of the concentrations presented in ng/ml [4].

2.4 Statistical Analysis

The collected data were analyzed using the Statistical Package for social sciences, version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp.) and Microsoft Office Excel 2010 for all variables and radar charts respectively. Results were expressed as mean±standard deviation (SD). The group means were compared by one-way ANOVA followed by Turkey's Post hoc test were performed to compare group means, and the Pearson correlation was executed for evaluation of the R (Correlation Coefficient) between respective parameters, with 95% Confidence-Intervals (CI). To maintain the quality of data, all anthropometric parameters were taken in duplicate with a high coefficient of reliability (R=0.99). The technical error of estimation of them was well within the limits [31].

4. RESULTS

We have studied 48 patients having PCOS [Age (years) range= (16-36) years, mean±SD= 22.17±3.81] and their age matched 45 healthy controls [Age (years) range= (16-35) years, mean±SD= 22.00±3.55]. The result (Table 1) illustrates the percentage of hirsutism, acne, AN, and alopecia among PCOS individuals respectively indicating hormonal imbalance.

Table 1: Clinical phenotypic classification of women having PCOS

Cutaneous manifestation	Sample size (n)	Percentage (%)
Hirsutism	48	71%(n=34)
Acne	48	40%(n=19)
AN	48	79%(n=38)
Alopecia	48	100%(n=48)

AN= Acanthosis Nigricans.

Inferential statistical analysis representing (Table 2) alternation in PI (P=0.000004, P<0.01) and BSA (P=0. 000443, P<0.01) in PCOS individuals in respect to controls indicating abnormal change in body fat level and metabolic mass in association with health risk.

Table 2: T-test between Ponderal Index (PI) and Body-Surface-Area (BSA) of control and PCOS patients

Parameters	Control n=45	PCOS n=48	Level of significance (Two-tailed)
PI (kg/m^3)	Mean±SD 13.25±2.68	Mean±SD 16.20±4.27	0.000004
BSA (m^2)* 10	Mean±SD 15.00±2.39	Mean±SD 16.16±1.66	0.000443

The percentage of total body fat distribution pattern and SF (%) of the Whole Body; (SWBF), Trunk; (STF), Arm; (SAF) and Leg; (SLF), and visceral area of control and PCOS patients were shown in the Radar chart (Figure 1).

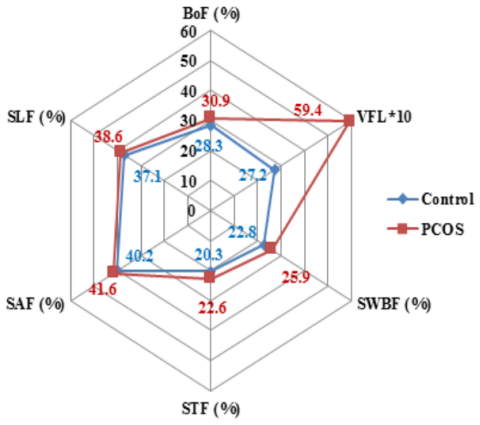
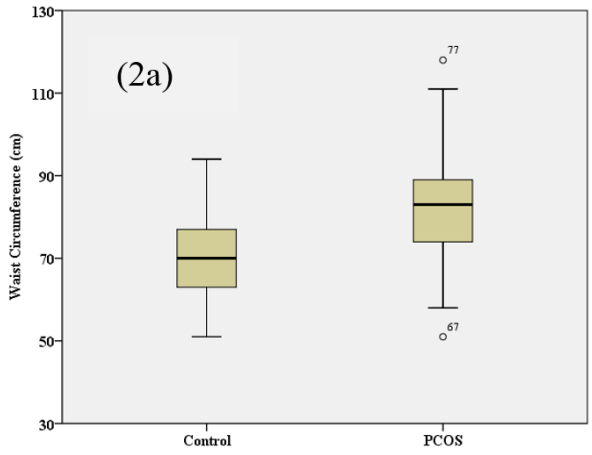
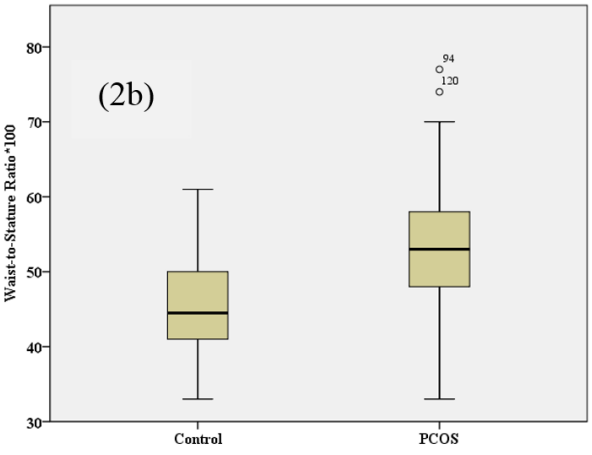


Figure 1: Radar chart of body fat, BoF (%) distribution pattern, Visceral Fat Level;VFL (%) and Subcutaneous Fat; SF (%) in Control subjects (Blue Color) and PCOS patients (Red Color): BoF (%): 28.3 and 30.9, VFL (%)*10: 27.2 and 59.4, and Subcutaneous Whole Body Fat; SWBF (%): 22.8 and 25.9, Trunk; STF (%): 20.3 and 22.6, Arms; SAF (%): 40.2 and 41.6 and Legs; SLF (%): 37.1 and 38.6.

Alternations in central obesity quantifying parameters like WC, WSR, BAI, and VFL in PCOS participants relative to control individuals were presented in box and whisker plots (Figure 2). Inferential statistics represented significant alternation (increase) of the parameters in PCOS individuals with respect to control {P< 0.01 level [P= (2a) WC; 0.000000, (2b) WSR; 0.000000, (2c) BAI; 0.000000 and (2d) VFL; 0.000000 respectively]} (Figure 2) showing excess fat accumulation in the patients.





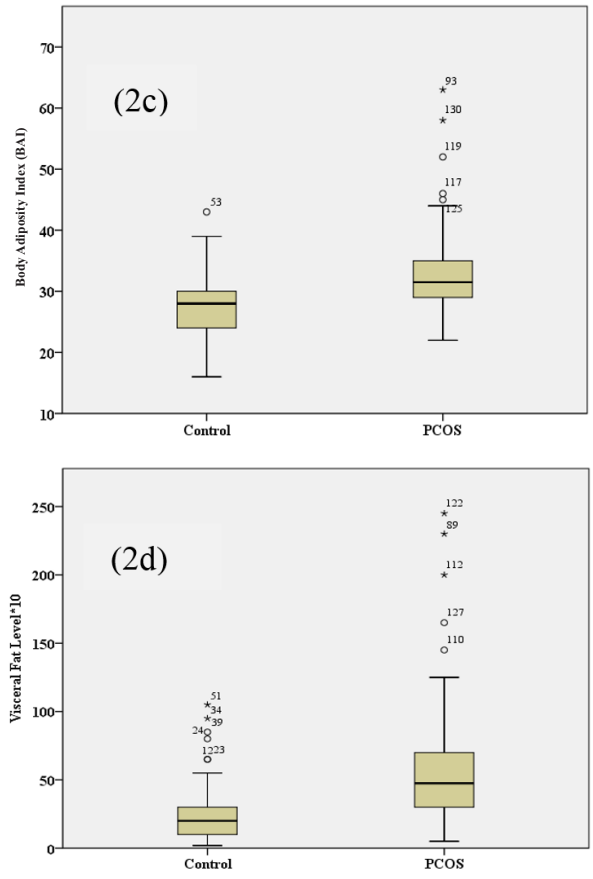
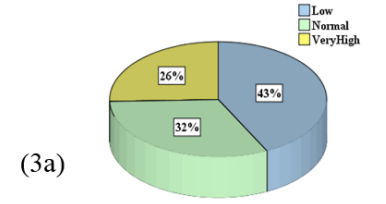
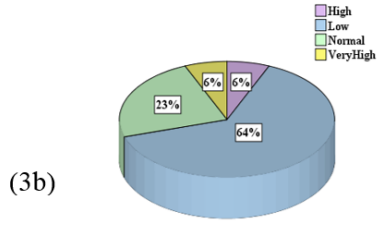


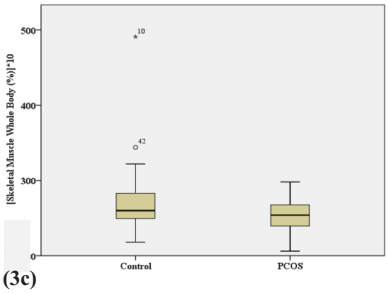
Figure 2: Altered in PCOS patients relative to control individuals- (2a) Waist Circumference; WC (2b) Waist-to-Stature-Ratio; WSR (2c) BAI; Body Adiposity Index and (2d) Visceral Fat Level; VFL. The simple boxplots are representing 25%, 50% (median) and 75% quartiles, whereas whiskers are representing minimum and maximum values of WSR. Significant alternation with respect to control P<0.01 level [P= (2a) 0.000000, (2b) 0.000000, (2c) 0.000000 and (2d) 0.000000 respectively].



(3a)



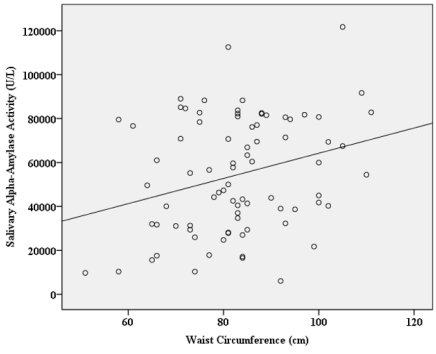
(3b)



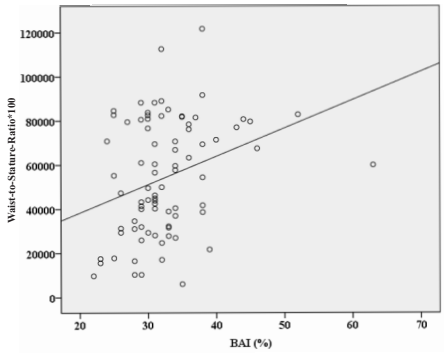
(3c)

Figure 3: Figure 3: Pie chart representing percentage of individuals with variation in level of skeletal muscle whole body; SMWB (%) level of (3a) control individuals and (3b) PCOS individuals. (3c) Significant alternation ($P< 0.05$, $P= 0.021$) in PCOS patients relative to control individuals. The simple box and whisker plots are representing 25%, 50% (median) and 75% quartiles, whereas whiskers are representing minimum and maximum values of SMWB (%).

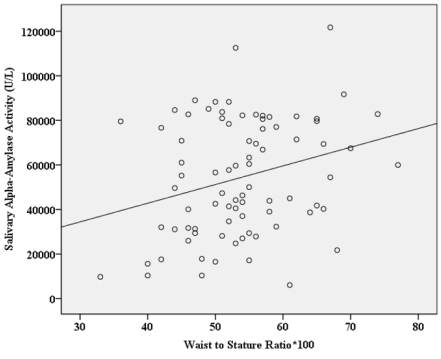
SMWB (%) plays an essential role in glucose uptake. In the present study percentage of individuals of control and PCOS group with variation in SMWB (%) were shown in pie chart (Figure 3) [control individuals- (3a) 43% (Low), 32% (Normal), and 26% (Very High) and (3b) PCOS individuals- 64% (Low), 23% (Normal), 6% (High) and 6% (Very High)]. The significant decreased amount of SMWB (%) in PCOS patients relative to control individuals was represented in box and whisker plot (Figure 3: (3c)). Inferential statistics represented significant alternation of the parameters in PCOS individuals with respect to control {$P< 0.05$ level, [$P= 0.021$]} (Figure 3: (3c)) PCOS individuals are susceptible to insulin intolerance. Figure 4 showed the correlation between stress biomarkers (SAA) activity and Salivary Cortisol Level) and central obesity quantifying parameters (WC, WSR and BAI). Bivariate analysis showed statistically significant positive correlation of SAA with WC [R= 0.277* and $P= 0.012$, $P<0.05$], WSR [R= 0.273* and $P= 0.014$, $P<0.05$] and BAI [R= 0.312** and $P= 0.005$, $P<0.01$] (Figure (4.1a), (4.1b) and (4.1c). Salivary cortisol was significantly negatively correlated with WC [R= -0.419* and $P= 0.041$, $P<0.05$] and WSR [R= -0.424* and $P= 0.039$, $P<0.05$] (Figure (4.2a) and (4.2b)).



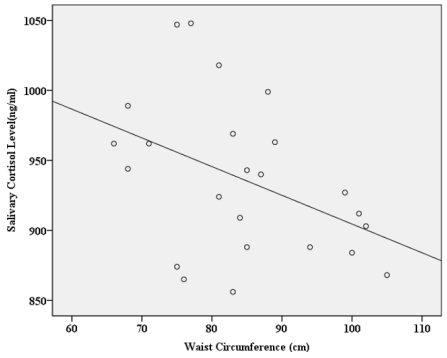
(4.1a)



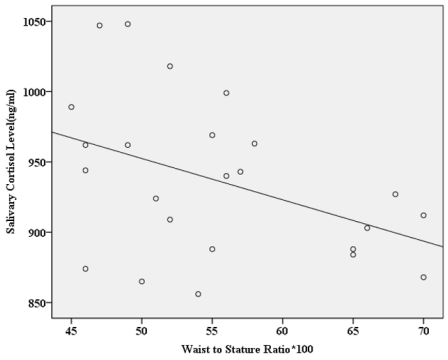
(4.1b)



(4.1c)



(4.2a)



(4.2b)

Figure 4: Correlation plot of relationship between Salivary α-Amylase, SAA activity and (4.1a) Waist Circumference; WC [R= 0.277* and *P*= 0.012, *P*<0.05]; (4.1b) Waist-to-Stature Ratio; WSR [R= 0.273* and *P*= 0.014, *P*<0.05] and (4.1c) Body-Adiposity-Index, BAI [R= 0.312** and *P*= 0.005, *P*<0.01], and Salivary Cortisol Level and (4.2a) WC [R= -0.419* and *P*= 0.041, *P*<0.05] and (4.2b) WSR [R= -0.424* and *P*= 0.039, *P*<0.05].

5. DISCUSSION:

Prevalence of cutaneous manifestation of hirsutism, acne, AN, and alopecia in PCOS individuals indicates probable deregulation in the functionality of endocrine and metabolic pathways. Previous studies stated that abnormal activity of hormones like testosterone, DHT, dehydroepiandrosteone sulfate (DHEA-S), insulin and insulin-like growth factor, and enzyme such as 5-α reductase may lead to hirsutism, acne, AN, and alopecia with a possible association with PCOS [8], [32]-[35]. In the present study (Table 1) the phenotypic expression is found to be a helpful parameter in analyzing hormonal and metabolic abnormality of PCOS. As PI is an indicator of leanness, it is more effective to predict obesity in adolescents which is considered to be a predominant factor of PCOS. In a previous study, it was evidenced that the tri-Ponderal mass index estimates body fat levels more accurately than BMI in non-Hispanic white adolescents [36]. In the previous study, we reported that BMI was statistically increased in PCOS patients relative to control individuals [4]. The present analysis statistically significant increase in PI (Table 2) further establish that variation in leanness in association with fat content is a useful predictive parameter of adiposity for PCOS patients. BSA is often used in preference to body mass (weight). For many clinical purposes, BSA is a better indicator of metabolic mass than body weight because it is less affected by abnormal adipose mass [37]. The present study statistically significant increase in BSA (Table 2) further establishes that variation in metabolic mass is a useful predictive parameter of adiposity for PCOS patients. Fat distribution is not a homogeneous phenotype, and the android pattern of body fat with disproportionate adipose tissue distribution in the abdominal visceral depots is a key feature and prevalent in 50%–60% of women with PCOS [19]. Central obesity is also highly prevalent in women with (PCOS) and strongly associated with MetS [38]. As reported earlier visceral fat could be a determinant of altered body composition in response to over expression of the stress axis [4], it was further compared with the distribution of SF content of PCOS and control group in Radar chart (Figure 1). Radar chart are a way of comparing multiple quantitative variables [39]. Figure 1 displays the BoF, VFL, and SF distribution (SWBF, STF, SAF, and SLF) radial or angular manner. These variables are represented on the axis from the same point and hence the relative position and the radially arranged axis are typically uninformative [40].

Alternation in central obesity quantifying parameters including WC, WSR, BAI, and VFL indicate deterioration in metabolic and endocrine functionality along with alarming health complications that may decrease reproductive fitness in association with IR and CVD [42]–[44]. Figure 2 represents a significant variation of the parameters in PCOS patients. SM plays an essential role in glucose absorption that prevents the expressivity of PCOS and a decrease in SM may enhance fat deposition association with PCOS tendency [45]. Figure 3 in the present study indicates variation in the level of SMWB and a decrease in PCOS.

Stress is considered to be an important factor in the pathogenesis of PCOS. Two neuroendocrine axes; HPA and SAM system are the primary regulators of the stress response [3], [4]. Salivary cortisol, a non-invasive index of free circulating levels of cortisol, is used as a measure of the HPA axis activation [4], [22]-[24] and SAA an enzyme that hydrolyzes starch in the oral cavity, is secreted by the parotid gland in response to adrenergic activity [29]. Several studies provided evidence that stimulation of the SAM system could significantly increase SAA levels rendering it a sensitive indicator of sympathetic activity [46]. The findings of the present study showed that (Figure 4) both the stress biomarkers are strongly associated with the central obesity indicating parameters and derived variables (SAA and (4.1a) WC [R= 0.277* and *P*= 0.012, *P*<0.05], (4.1b) WSR [R= 0.273* and *P*= 0.014, *P*<0.05] and (4.1c) BAI [R= 0.312** and *P*= 0.005, *P*<0.01], and Salivary Cortisol Level and (4.2a) WC [R= -0.419* and *P*= 0.041, *P*<0.05] and (4.2b) WSR [R= -0.424* and *P*= 0.039, *P*<0.05]). These observations emphasize the importance of assessment of physical parameters and the necessity for making an anthropometric profile to set a criterion for the diagnosis of PCOS.

COVID-19 pandemic era with the changing epigenetic factors; specifically diet, sedentary lifestyle, and less sun exposure could play an influential role in the crosstalk with the multigenic trait responsible for PCOS and adversely affect the expression of the other associated metabolic abnormalities. In present new-normal an interrelated multidisciplinary study in terms of socioeconomic conditions, ethnicity and demographic genetic features related to PCOS, the severity of clinical manifestations, changing educational scenario and peer pressure, and screen time, as well as stress biomarkers, is on an urgent need for the betterment of PCOS population.

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