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Original article

Association of ageing and PCOS women's metabolic turbulence: A cohort study in hospitals of Faisalabad, Pakistan

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Background: Ethno-demographic data on polycystic ovary syndrome (PCOS) is still prone to contentious debate since heterogeneity amid PCOS women of different regions.

Objective: To study the impacts of ageing on endocrinopathy of metabolic disorders (MetS) among PCOS women and cardiovascular risk (CVR).

Methods: In the current study, PCOS women (n = 500) with age range of 15 - 45 years were recruited from Faisalabad region of Pakistan and their reproductive history with brief demographic data were recorded through interview questionnaire while medical assessments were carried out under protocol. Following categorization of five age groups, statistical significance was determined through analyses of variance and regression analysis.

Results: Mean number of menstrual cycles per year, luteinizing hormone: follicle stimulating hormone ratio, hirsutism score declined significantly with advancing age of PCOS women ($P < 0.05$). While unprecedented age related progression was ensued in cycles' duration, bleeding days after menarche onsets, polycystic ovary morphology (PCOM) components including ovarian volume, diameter and number of follicles. Among PCOS women with MetS, mean fasting blood glucose and waist to hip circumference ratio, body mass index, and blood pressures were examined the highest in older women but inverse relationship of high density lipoprotein (HDL) was noticed with progressive age groups ($P < 0.01$). Reciprocal attenuation in stillbirth and inflation in conception rate were also evident among progressive age groups accordingly. Similarly, Framingham points and CVR_{10} was significantly the highest (1.5%) among older age groups PCOS women with increasing age conformity ($P < 0.05$).

Conclusion: Ageing among PCOSs, unequivocally, exacerbated the endocrinopathy while synergetic metabolic abnormalities have transcended impacts on cardiovascular risks among older aged PCOS women.

Keywords: Cardiovascular diseases, framingham risk scoring system, hyperandrogenism, metabolic syndrome, polycystic ovary syndrome.

Among all females reproductive disorders polycystic ovary syndrome (PCOS) is the most intensely studied because of its controversial nature of manifestation altered with ethnicity, body weight, age and even clinician expertise that sets the threshold diagnostic line.[1] Besides diagnostic criterion history [2, 3] current combination of hyperandrogenism, anovulation and polycystic ovary morphology (PCOM) symptoms are commonly used to diagnose PCOS.[3] While the androgen excess criteria have a priority place among others [4, 5] since more likely major reason of co-morbidities including metabolic syndrome. However, without hyperandrogenism PCOS has already been evidenced the least severe PCOS phenotypes.[5] While, senescence especially in women, with elegant reproductive endocrine attributes, likely has more potential to exacerbate the existing endocrinopathy and co-morbidities in PCOS subjects.[6] Moreover, the importance of ethnic variability in PCOS manifestation has already been emphasized in several reports.[7, 8] Asian women with unprecedented polycystic ovary endocrinopathy and metabolic outcomes have also implication to revisit the PCOS diagnostic criteria and criterion for heterogeneity in phenotypes.[9] However, several studies implicated endocrine and metabolic complications in PCOS subjects are inevitable with age [6, 10, 11] emphasizing on the importance of age associated ovarian functions alteration with metabolic

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disorder (MetS) expression including cardiovascular risk (CVR).[7, 12] Obesity and cardiometabolic risk association were found more severe among women than men, but in both genders this equivocal exacerbation unlike attenuation was also noticed with increasing age [1, 13] and hence hitherto controversial. Whereas Macut D, *et al*. [14] examined 1.6% CVR among Serbian PCOS women without transcended age rendered a chasm of information. However very lately a retrospective study indicated the higher probability of general CVR was in both adolescent and adult PCOS black women than white thence emphasized on importance of longitudinal study addressing the impact of PCOS and race on CVR in women.[15] Therefore, the current study is designed to observe age related PCOS manifestation among women from Faisalabad, Pakistan. The objective of this study was to examine the impacts of ageing on cardiovascular risk in commencing 10 years (CVR_{10}) through Framingham risk scoring system (FRSS) among PCOS women with MetS.

Materials and methods

The present cohort study was carried out on PCOS women of reproductive age following signed consent recruited from hospitals in Faisalabad, District Head Quarter Hospital, Allied Hospital, and gynecology clinics, during the period of August 2014 to February 2015. Recommendation was obtained following submission of research proposal with methodology, revised following STROBE guidelines for cohort study, to institutional to Institutional Review Board for human research. Briefly, questionnaire, prepared for face-to-face interview, had two sections, one was socio-demographic information and other was compendium of question related to reproductive history of recruited women.

Participant's criteria

Geographically, Faisalabad city is divided into sub-regions and marked the gynecological centers and hospitals in each for cluster sampling. Subsequently, questionnaire and probability of PCOS patients visited was discussed with gynecologist in each unit. Finally, randomly selected two to three clinics and/or hospitals from each sub-region were subjected for recruitment of women and interview. In this study, positive diagnosis of PCOS (n = 500) [(on the basis of two amid three diagnostic parameters) oligomenorrhoea/ oligo-ovulation Oligomenorrhea was distanced as having fewer than eight menstrual cycles/year, or when the duration of a cycle exceeds 35 days. Amenorrhea was ascertained by the absence of three to six consecutive menstrual cycles, or four or fewer menstrual periods per year [16], clinical or biochemical hyperandrogenism and polycystic ovaries on ultrasound, ≥ 12 follicles in each ovary, measuring 2 – 9 mm in diameter and/or increased ovarian volume of each ovary 10 ml] [17] while metabolic syndrome (MetS) as described by World Health Organization (WHO) [18] high-density lipoprotein (HDL) < 50 mg/dL, waist-to-hip ratio > 0.85 and fasting blood glucose > 110 mg/dL) was mandatory for participation of females. However women with incomplete sought metabolic profile, inherited disorders, Cushing syndrome, insulin resistance, congenital adrenal hyperplasia and ovarian or adrenal tumor were not included in this study.

Reproductive history and clinical evaluations

Reproductive cyclic history was recorded by sets of question subjected menarche and after marriage cyclic changes like age at both stages with menses duration and menstrual cycles/year.[16] Ultrasonographic scans of right and left ovaries with volumes, follicular number and diameters were also recorded as described by Balen AH, *et al*. [19] Finally physical examination of body mass index (BMI), blood pressure, modified Ferriman-Gallwey (F-G) hirsutism score as practiced by Wijeyaratne CN, *et al.* [17] and Unluhizarci K, *et al*. [20], and waist and hip circumference with their ratio as outlined by Balen AH, *et al.* [19] were also included in the current study. Fasting blood glucose, total cholesterol, low density lipoprotein (LDL) and HDL cholesterol, triglyceride and gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations in serum samples from each participant was also determined as alluded in standard assay protocols. While FRSS was also employed to estimate 10-year cardiovascular risk percentage among women considering the metabolic attributes points including age, total cholesterol, HDL, smoking status and systolic blood pressures in all participants as described in National Institutes of Health guidelines.[21] Fertility related questions like the number of miscarriages and live births were also included in questionnaire.

Statistical analysis

The data of 500 subjects sorted into five age groups of youngest/ adolescent (< 19 years), younger

(20 - 26 years) young (27 - 33 years) old (34 - 40 years) and older (> 41 years) women with diagnosed PCOS. Data were expressed as mean ± standard error of the mean (SEM). Age associated changes in cyclic, gondotropin and F-G-hirsutism score, ovarian scans attributes as well as metabolic malfunctioning recordings were also determined employing regression analysis with R^2 values and regression line equation reflecting the mean values deviation from alluded line. However, the one-way analysis of variance (ANOVA) was also applied to ascertain the difference in parameters from each group and Tukey's post hoc test also carried out to determine the exact significant difference between the means of each parameter from two different age groups. All these statistical analysis were carried out on software Graphpad Prism Version 6.0.

Results

This study was designed to address the effects of age on metabolic disorder manifestation in PCOS women from hospitals located in Faisalabad city of Pakistan. An overview of reproductive history of recruited subjects (n = 500) is presented in Table 1.

Table 1. An overview of reproductive history of PCOS subjects (n = 500) recruited from hospitals in Faisalabad.

	Mean ± SEM	
Age (yr)	25.83 ± 0.32	(500)
Body weight (kg)	73.69 ± 0.65	(500)
Reproductive cycles		
Age at Menarchea (yr)	13.90 ± 0.05	(500)
Menstural dischage (day)	6.44 ± 0.11	(500)
Number of Menstural cycles/year	12.48 ± 0.17	(500)
Age at Marriage (yr)	22.39 ± 0.24	(414)
Menstural cyles (day)	72.03 ± 2.34	(500)
Number of Menstural cycles/year	6.57 ± 0.20	(498)
Endocrinology		
LH	19.94 ± 0.18	(500)
FSH	7.25 ± 0.07	(500)
LH:FSH	2.83 ± 0.03	(500)
Hirsutism score	6.95 ± 0.16	(499)
Ultrasonographic scan		
Right ovary		
Volume (cm^3)	10.55 ± 0.17	(500)
Number of Follicles	13.15 ± 0.09	(500)
Diameters of Follicles (mm)	4.85 ± 0.04	(500)
Left ovary		
Volume (cm^3)	13.03 ± 2.49	(500)
Number of follicles	12.83 ± 0.26	(500)
Diameters of Follicles (mm)	4.88 ± 0.04	(500)
Metabolic components		
Blood pressures		
Systolic (mmHg)	123.87 ± 0.61	(500)
Diastolic (mmHg)	84.48 ± 0.39	(500)
Lipid assessment		
BMI (kg/m^2)	29.19 ± 0.26	(500)
LDL (mg/dL)	176.41 ± 1.09	(148)
HDL (mg/dL)	31.87 ± 0.29	(148)
Total Cholestrol (mg/dL)	205.43 ± 2.35	(148)
Triglyceride (mg/dL)	154.58 ± 2.47	(134)
Waist Circumfrence (cm)	113.79 ± 1.35	(500)
Hip Circumfrence (cm)	124.14 ± 1.40	(500)
Waist: Hip ratio	0.97 ± 0.06	(498)
Fasting Blood Glucose Level (mg/dL)	181.88 ± 1.81	(161)
Fertility potential		
Age at diagnosis (yr)	30.52 ± 0.45	(134)
Age at first conception (yr)	25.64 ± 0.31	(135)
Number of live births	2.67 ± 0.20	(134)
Number of miscarriages	0.83 ± 0.07	(135)

Note: SEM is standard error of mean and values in parenthesis are the number of PCOS subjects

Menstrual cycle alteration

Mean number of menstrual cycles per year at menarche and after marriage within five age including youngest/ adolescent (< 19 years, n = 115), younger (20 - 26 years, n = 161), young (27 - 33 years, n = 139), old (34 - 40 years, n = 78) and older (> 41 years, n = 7) age groups are shown in Figure 1, top left panel. Mean number of cycles/year during both times carried considerably (F = 80.45 at menarche, F = 11.13 after marriage, $P < 0.0001$) in PCOS subjects from five age groups. Predominantly, old aged females (34 – 40 years) had the fewest mean cycles/year ($P < 0.001$, 3.0% decline from youngest females), after the onset of first period, than other age females. While the mean numbers of cycles/year at menarche ensued from females of each age group were diverted from regression line though non-significantly progressive with increasing age groups. However mean number of cycles/year after marriage was considerably ($P < 0.01$) declining with increasing age groups with values closer to regression line. PCOS females of old age (34 - 40 years) had significantly ($P < 0.01$, 47.0% decline from youngest females) fewer mean number of cycles/year after marriage than other age females. But the fewest cycle/ year (3.14 ± 0.74/year, 63.0% decline from youngest females) was recorded in females from older age groups (> 41 years) when compared to old age females.

Other menstrual history characteristics including mean age, menses duration (day) at menarche and cycle's duration (day) after marriage of PCOS subjects from five different age groups are shown in Table 2. Regression analysis ensued no effect of age over the age of menarche and bleeding duration at menarche but mean menses duration in cycles were noticeably the longest in younger PCOS subjects compared to other age groups except older aged women (Figure 1A) ($P < 0.01$). However, age at marriage also declined while mean days of cycles duration increased appreciably with increasing age of PCOS subjects ($P < 0.05$). Whereas the longest mean duration of cycles was found significant from old (74.0% increased from youngest females) and older age groups (161.0% increased from youngest females) when compared to other lower age groups ($P < 0.05$).

Gonadotropins and hirsutism

There is no appreciable difference among the age groups of PCOS when F-G hirsutism score and LH: FSH ratio data statistically tested employing ANOVA and regression analyses (Table 2). However, gonadotropins ratio was ensued the highest in youngest subjects but slight drop was also visible among all age groups (2, 5, 3 and 13.0% decline in younger, young, old and older age groups respectively). Whereas the least mean FG hirsutism score was estimated in subject of older age group and the highest in youngest females. Moreover, no significant ($P > 0.05$) decline in score was also ascertained with progressive age of subjects (10, 5, 12 and 44.0% decline in younger, young, old and older age groups, respectively).

Ultrasonographic ovary scan

Mean volume of ovary, diameter of follicles and number of follicles from right ovary of PCOS subjects were significant difference ($P < 0.001$) among the age groups. Mean volume of ovary [47.0% (old aged) and 91.0% (older aged) higher from youngest females] and diameter of follicles [11.0% (old aged) and 36.0% (older aged) higher from youngest females] from old and older age groups was significantly ($P < 0.05$) greater than lower age groups. But the mean number of follicles of right ovary from young aged females were significantly higher (6.0%) ($P < 0.05$) than youngest females. However, the slight progression in mean ovarian volume and diameter of follicles, from both right and left, with increasing age while obvious deviation between calculated and expected in values ensued from young (27 - 33 years) and old age (34 - 40 years) groups (Figure 1B, D & F). There was no significant difference in number of follicles of both ovaries from all age groups and without any statistical effect of age on mean numbers. However 1.0%, 4.0% and 7.0% decreases were also recorded in the mean number of follicles in right ovary from older aged PCOS subjects compared to youngest, young and old aged females, respectively.

Blood pressure and body mass index (BMI)

A considerable increase in mean systolic and diastolic pressure and mean BMI was ascertained as age progressed without any noticeable divergence of means from regression line (Figure 1C & E) ($P < 0.01$). However, both pressures and BMI from old age groups (34 - 40 years) were significantly ($P < 0.05$) higher than lower age groups including < 19 (18, 21 and 20.0% increase in systolic, diastolic pressures and BMI, respectively), 20 - 26 (10, 14 and 10.0 % increase in systolic, diastolic pressures and BMI respectively) and the groups of 27 - 33 years old (7, 11 and 5.0 % increase in systolic, diastolic pressures and BMI respectively). While there was no significant difference in both pressures and BMI from both age groups, 34 - 40 years and > 41 years old ($P > 0.05$).

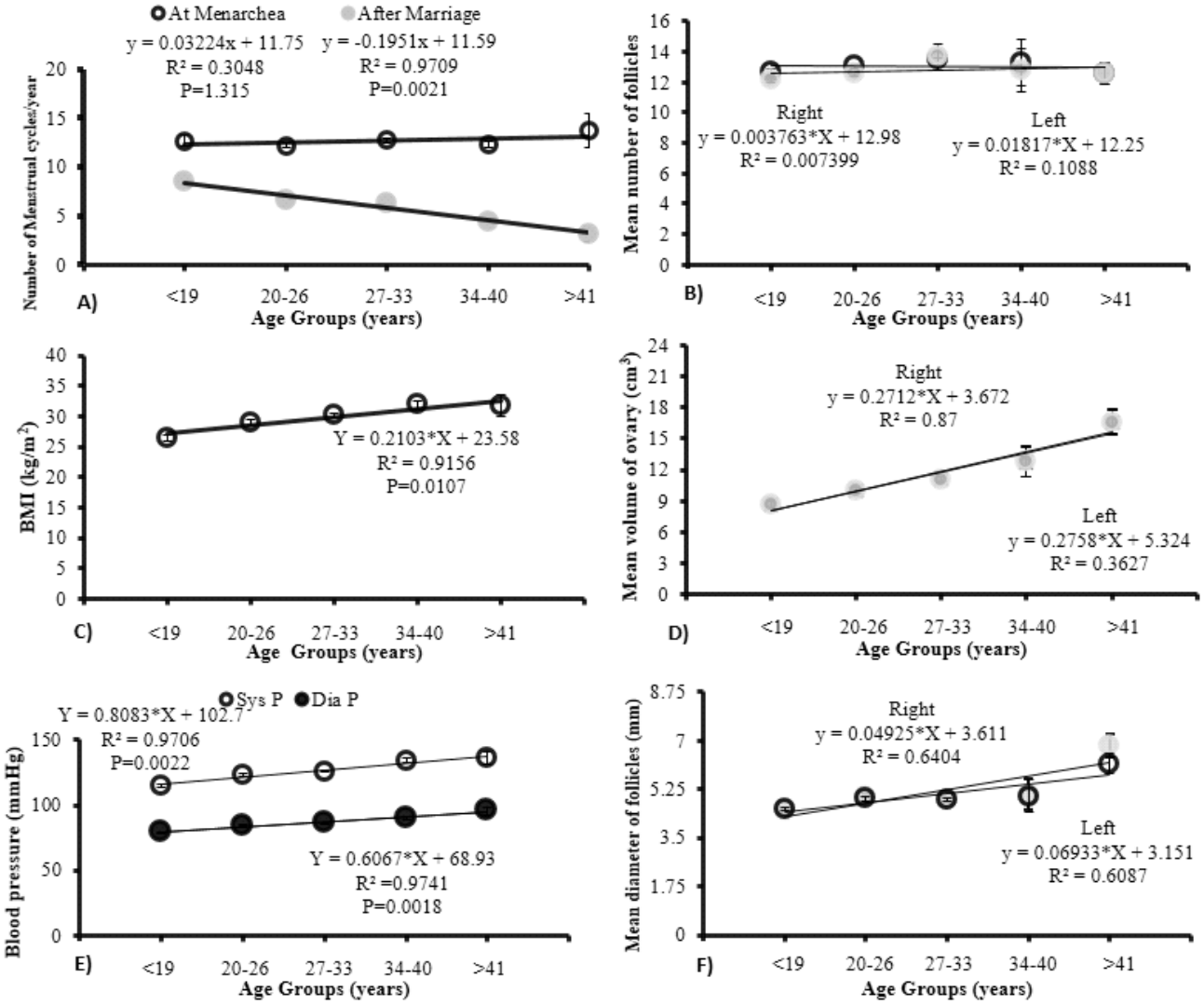

Figure 1. A) Age corresponding changes in mean number of menstrual cycles/ year recorded at menarche and after marriage of PCOS women. C) Positive trend of mean BMI and E) mean systolic and diastolic blood pressures ascertain positive trend towards advancing age groups. While B), D) & F) presenting the mean number of follicles in right and left ovary, mean volume of both ovaries and mean diameter of antral follicles in both ovaries respectively recorded in PCOSs from five different age groups. However, unprecedented increase in ovarian volume with increasing age of PCOS women, though stable number of follicle pool was also ascertain in all age groups.

Lipid profile

Mean total cholesterol, LDL and triglyceride serum levels in PCOS subjects were neither statistically showing any significant association with increasing age nor considerably different from each another age groups (Table 2) ($P > 0.05$). While mean waist circumference and hip circumference (WC and HC) have appreciable association with progressive age groups ($P < 0.01$) and means were not deviated from regression line. Whereas the old aged subjects (34 – 40 years) had significantly higher both circumferences compared to young (20 - 26 years) (10.0% WC and 15.0% HC higher) and younger (< 19 years) (22.0% WC and 25.0% HC higher) aged subjects ($P < 0.001$). Moreover, older subjects had the highest both circumferences (26.0% WC and 27.0% HC higher than youngest).

WHO criteria for metabolic syndrome

Mean HDL and mean fasting glucose had no association with the progressive age groups while the divergence of mean waist-to-hip ratio from regression line was ascertained the unlike association between age and ratio (Figure 2A). However, the mean fasting blood glucose of PCOS subjects from older age group was considerably higher than the younger (29.0%), young (30.0%) and old age groups women (18.4%) ($P < 0.05$).

Table 2. Menstrual history including mean age and discharge duration at menarche; mean age and cycles duration (day) after marriage, LH: FSH ratio, mean hirsutism, lipid profile, waist and hip circumferences, and fertility potential related parameters of PCOS women from five different age groups.

	Age groups (yr)						
	< 19	**20 - 26**	**27 - 33**	**34 - 40**	**> 41**	**R^2**	***P*-value**
Age at menarche (yr)	13.89±0.11 (115)	13.80±0.09 (161)	13.91 □ 0.10 (139)	14.06±0.14 (78)	14.71±0.36 (7)	0.643	0.10
Bleeding duration (day)	7.86±0.33 (115)	6.04±0.14^{a**} (161)	5.99±0.17^{a**} (139)	5.96±0.19^{a**} (78)	6.79±1.13 (7)	0.205	0.44
Age at marriage (yr)	15.72±0.21 (107)	22.05±0.31^{a*} (88)	25.22±0.22^{ab*} (134)	26.50±0.36^{abc*} (78)	25.86±1.08^{ab*} (7)	0.793	0.04*
Cycles duration(day)	55.65±1.85 (115)	64.06±1.61 (161)	77.10±7.06 (139)	96.99±4.86^{abc*} (78)	145.70±22.13^{abc*} (7)	0.865	0.02*
LH:FSH	2.91±0.05 (115)	2.84±0.06 (161)	2.76±0.06 (139)	2.82±0.08 (78)	2.53±0.20 (7)	0.692	0.66
Hirsutism score	7.50±0.35 -115	6.72 □ 0.27 -160	7.09±0.29 (139)	6.62±0.38 (78)	4.14±0.40 (7)	0.081	0.10
Total Cholestrol (mg/dL)	200.30±7.91 (6)	201.70±5.86 (26)	206.80±3.59 (54)	204.60±4.07 (58)	231.50±6.44 (4)	0.624	0.11
LDL (mg/dL)	180.20±9.24 (6)	175.20±2.81 (26)	176.90±1.81 (54)	175.50±1.58 (58)	185.80±2.14 (4)	0.149	0.52
Triglyceride (mg/dL)	156.00±10.18 (6)	151.50±6.18 (26)	151.10±4.20 (50)	159.30±3.60 (50)	158.50±41.50 (4)	0.268	0.37
WC (cm)	101.90±3.36 (115)	112.70±2.23^{a*} (161)	118.10±2.21^{a*} (139)	124.50±2.95^{ab**} (78)	128.90±8.14 (7)	0.98	0.001***
HC (cm)	111.70±3.03 (115)	121.10±2.14 (161)	128.60±1.98^{a*} (139)	139.30±4.72^{ab**} (78)	142.00±6.97 (7)	0.983	0.001***
Age at diagnosis (yr)	- 0	25.77±1.40 (13)	28.09±0.37 (56)	32.93±0.67^{bc*} (58)	38.71±0.71^{bcd*} (7)	0.965	0.018**
Age at first conception (yr)	- 0	23.62±0.94 (13)	24.80±0.56 (56)	26.90±0.35^{bc**} (59)	26.29±1.09 (7)	0.795	0.108
Number of live births	- 0	1.46±0.27 (13)	2.13±0.16 (56)	3.17±0.41 (58)	5.00±0.31^{bc***} (7)	0.944	0.028*
Number of miscarriages	- 0	0.85±0.15 (13)	1.02±0.12 (56)	0.70±0.11 (59)	0.29±0.18 (7)	0.676	0.178

Note: Values are Mean ± SEM and age groups are in years and numbers of PCOS women from each age group are in parenthesis, while symbols yr, WC, HC, R^2 and *P* are year, waist circumference, hip circumference, coefficient of regression and probability respectively. However at values superscripts alphabets, a, b, c and d are the significant difference, employing Tukey's test, from < 19 years, 20 - 26 years, 27 - 33 years, 34 - 40 years and > 41 years age groups, respectively. $P < 0.05$*, $P < 0.01$**and $P < 0.001$***

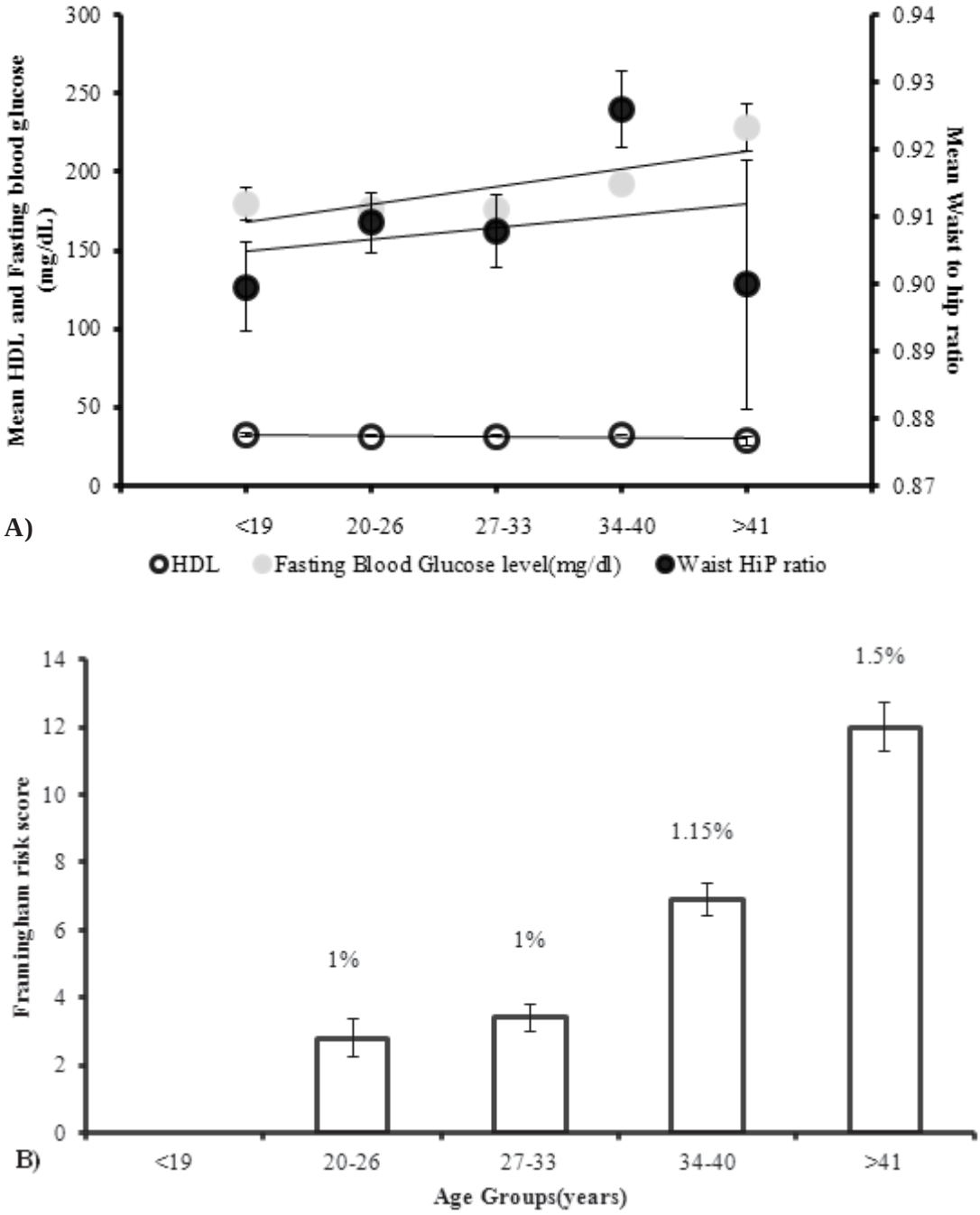

Figure 2. A) Mean HDL (mg/dL) and fasting blood glucose (mg/dL) (primary axis) had non-significant decrement and increment ($R^2 = 0.553$, $P = 0.150$ and $R^2 = 0.631$, $P = 0.109$ respectively), with advancing age of PCOS women. Whereas B) mean waist circumference to hip circumference ratio (secondary axis) had shown fluctuation at each age group point though comply futile association with increasing age. However, a successive elevation trend was ensued in FRS points in each age group of PCOS women and was maximum in older PCOS women. While corresponding to FRS, CVR_{10} (percentages at top of each bar) was also shown the same age associated trend with maximum cardiovascular diseases risk PCOS women from old (34 - 40 years) and older (> 41 years) age groups.

Fertility potential

Mean age at diagnosis and at first conception of PCOS subjects from old age group was considerably higher than younger and young aged subjects (Table 2) ($P < 0.05$). Whereas appreciable regression line was ascertained the progressive association between the mean age of diagnosis and age groups ($P < 0.05$). Similar progressive association was also resulted between mean number of live births and age groups. Mean number of live births among older aged PCOS females was significantly the highest (242.0%) compared with young and younger age groups of females ($P < 0.01$). However, no significant difference existed between the age groups when analyzed from means number of miscarriages, even the means were not associated with progressive regression line.

Framingham risk scoring system (FRSS)

Mean Framingham risk scores for cardiovascular risk percentage estimated in females from old and older age groups were found considerably higher (327.0%) than the young and younger age groups (Figure 2B). While the positive association ($R^2 = 0.8968$) was also estimated between scores and increasing age of females. Likewise the older aged females were found most susceptible to cardiovascular diseases (CVR_{10} = 1.5%) than the other groups' females.

Discussion

The findings of the study not only revealed the severity of clinical features of PCOS subjects with increasing age but also implicate parallel association with MetS and Framingham CVR_{10}. It is very rational that older women are more prone to exacerbation of endocrinopathy and infertility associated with metabolic disorders especially cardiovascular risks. Hence, a diagnostic criterion for PCOS is more likely to be reviewed accordingly with increasing age of women even from every region of world. Likewise, oligoamenorrhea and amenorrhea had already been evident common among adolescent ages–however regularity in menstruation was endeavored among older aged women with PCOS.[22] Unprecedented ovarian volume and number of follicles were also marred with senescence of women, either PCOS or normal.[23] Similarly, deficit in insulin action may likely strengthen the expression of metabolic disorders and endocrinopathy scaffolding menace fertility.

While PCOS diagnostic importance Anti-Mullerian hormone (AMH) serum levels [24] and its correlation with the number of menstrual cycles per year subsequently explain the unlike irregular cycles in PCOS subject during older age.[25] Similar, studies revealed conversion of 20.0% anovulatory PCOS patients into ovulatory after five years follow up at the mean age of 45 years and justified by follicular loss with ovarian ageing.[26] Current study's results were also corroborated with the same notion of significant ($P < 0.01$) decline (47.0%) in the mean number of menstruation per year at old (34 - 40 years) age compared to youngest females after marriage. Moreover, mean number of cycles per year also gradually dropped 63.0%, 52.0% and 50.0% among females in older age group (> 41 years) from youngest, younger and young age groups respectively. PCOM examinations of the current study also shows the same diminishment in follicular pool. This decline in cycles per year and follicular pool in ovaries with increasing age was likely normalizing the menstruation and ovarian ageing. Hence, increased potential of conception was also observed strengthen in older PCOS. Moreover four years delay in menopause was also ascertained lately among PCOS than normal.[27] While in contrast, ovarian volume was resulted positively associated with advance age contradicted from the findings of Carmina E, *et al.*[28]

A comprehensive study by Bili H, *et al.*[11] revealed the exponential decrease in LH, testosterone and LH to FSH ratio recorded from old PCOS women when compared to younger women. In addition, obese oligoamnorrhoeic hirsute patients had higher serum testosterone compared to patients with normo-ovulatory hirsutism[28] which indicated a relation of hyperandrogenism and women cyclicity. However, inverse relationship between serum androgens and age had already been documented among the normal women at their reproductive age and above 45 years age even stable serum LH was reckoned.[29] However, Moran C, *et al.*[30] resulted 25.0 – 30.0% deflation in serum androgens in PCOS women of 22 to 45 years old.

Although the current study ensued no significant difference of mean hirsutism score and LH: FSH ratio in PCOS females from five age groups but slight decline in score and ratio was noticed with increasing age (especially, 5.0% in older PCOS women). However, blood sampling time, pulsatile nature of LH secretion and immunoassay may alter LH and futility

of negative relationship with advanced age [11] but age mediated decline in hirsutism has already evidenced. [31]

Current PCOM results also revealed more responsiveness of right ovary to age related changes where mean ovarian volume and diameter of follicles presented positive association with increasing age of PCOS females while mean number of antral follicles were reciprocal to ageing.

Metabolic syndrome related characteristics, mean HDL, fasting blood glucose and waist-to-hip ratio had futile association with progressive age. However, the fasting blood glucose was recorded appreciably higher in older PCOS females. Current findings also revealed the implication of high fasting glucose levels mediated amelioration of insulin resistance and inflated ovarian insulin receptors responses justifying the hyperandrogenism in older PCOS women. [11, 32] Several studies have documented negative effect of androgens on lipid metabolism especially on HDL in normal both men and women [33] since increased catabolism of HDL though hepatic endothelial triglyceride lipase [34] also augmented by androgens.[35] One of the recent studies also revealed the greater prevalence of black PCOS women were with the low high-density lipoprotein and high blood glucose. Moreover, black adolescent and adult PCOS were found more prone (relative risk 2.65 and 1.44, respectively) to MetS than white PCOS subjects. [15] In current study, similar decline in HDL was observed in older PCOS female (13.0%) than younger likely orchestrated with hyperandrogenism. However, the mean total cholesterol progressively increased in advanced age of PCOS females which likely confirmed the establishment of hyperlipidemic condition in metabolic syndrome.

Testosterone and BMI positive correlation [31] and age driven inflation in obesity [36] were the hallmark findings to ascribe the decrease incidents of insulin resistance among younger PCOS women. [37] While Carmina E, *et al.* [28] have revealed the futile influence of ageing on the insulin sensitivity index however metabolic abnormalities and waist circumference increased. Similarly, in the current study, mean BMI, blood pressure, WC and HC of all PCOS females ascribed appreciative link with ageing especially the females of old age group.

Metabolic syndrome is the compendium of cardio-metabolic factors; hence, strengthens the incidence of cardiovascular diseases [38] in both genders. Moreover, androgen excess, a hallmark of PCOS, was also found a major cause of risk of coronary artery diseases. [39] Onslaught of hyperandrogenesim on ovarian structure and functions enhanced cardiovascular risk factors [40] especially in PCOS women. [14] In similar manner, the current study also revealed age related exacerbation in risk factors (1.5% CVR_{10} was recorded in older age PCOS women). Whereas, the FRSS estimations were considering metabolic factors and age of PCOS women revealed the same association of women senescence as examined other parameters like obesity, hypertensiveness and abnormal lipid profile.

However, the ovarian ageing mediated normalization in menstrual cyclicity is also evident except increased ovarian volume which was more likely due to more oocytes recruitment in each menstrual cycle abnormally and hyperandrogenism. While MetS components, waist to hip ratio and fasting blood glucose levels had futile association with advance age. Because of limited resources and facilities it was not possible though other assessments for diabetes including, oral glucose tolerance test, microalbuminurea and HbA1c were more likely suitable to explain the significant association with age. However ageing impacts were also ascertained unequivocally other metabolic dysfunctions related parameters including blood pressure, lipid profile and BMI. Moreover old and older PCOS women were also found more susceptible for cardiovascular disease determined CVR_{10}.

Conclusion

Examination of endocrinopathy and PCOM parameters in current study are evidenced inevitable for PCOS diagnosis and are concordant with the recent studies on population from several regions of world.

Conflict of interest

The author has no potential conflict of interest to disclose.

References

1. Hsu MI. Clinical characteristics in Taiwanese women with polycystic ovary syndrome. Clin Exp Reprod Med 2015;42:86-93.
2. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome towards a rational approach. In: Dunaif A, Glvens JR, Haseltine FP,

Marriam, GR, editors. Polycystic ovary syndrome. Boston: Blackwell Scientific; 1992. p. 377-84.
3. The Amsterdam ESHRE/ASRM- Sponsored 3rd PCOS Consensus workshop group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod 2012;27:14-24.
4. Rosenfield RL, Ehrmann DA, Littlejohn EE. Adolescents polycystic ovary syndrome due to functional ovarian hyperandrogenism persists into adulthood. J Clin Endocrinol Metab 2015;100:1537-43.
5. Wang F, Pan J, Liu Y, Meng Q, Lv P, Qu F, et al. Alternative splicing of the androgen receptor in polycystic ovary syndrome. Proc Natl Acad Sci USA 2015;112:4743-8.
6. Tsikouras P, Spyros L, Manav B, Zervoudis S, Poiana C, Nikolaos T, et al. Features of Polycystic Ovary Syndrome in adolescence. J Med Life 2015;8: 291-6.
7. Wang FF, Pan JX, Wu Y, Zhu YH, Hardiman PJ, Qu F. American, European, and Chinese practice guidelines or consensuses of polycystic ovary syndrome: a comparative analysis. J Zhejiang Univ Sci B 2018; 19:354-63.
8. Wei HJ, Young R, Kuo IL, Liaw CM, Chiang HS, Yeh CY. Prevalance of insulin resistance and determination of risk factors for glucose intolerance in polycystic ovary syndrome: a Cross-sectional study of Chinese infertility patients. Fertil Steril 2009;91:1864-8.
9. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010;25:544-51.
10. Hsu MI. Changes in the PCOS phenotype with age. Steroids 2013;78:761-6.
11. Bili H, Laven J, Imani B, Eijkemans MJ, Fauser BC. Age-related differences in features associated with polycystic ovary syndrome in normogonadotrophic oligo-amenorrhoeic infertile women of reproductive years. Eur J Endocrinol 2001;145:749-55.
12. Zhang HY, Guo CX, Zhu FF, Qu PP, Lin WJ, Xiong J. Clinical characteristics, metabolic features, and phenotype of Chinese women with polycystic ovary syndrome:a large-scale case-control study. Arch Gynecol Obstet 2013;287:525-31.
13. Wakabayashi I. Age-dependent influence of gender on the association between obesity and a cluster of cardiometabolic risk factors. Gend Med 2012;9:267-77.
14. Macut D, Bozic I, Popovic B, Bogavac T, Petakov M, Ognjanovic S, et al. Metabolic syndrome indices and Framingham risk scoring in women with polycystic ovary syndrome. Endocrine Abstracts 2010;22:P473.
15. Hillman JK, Johnson LN, Limaye M, Feldman RA, Sammel M, Dokras A. Black women with polycystic ovary syndrome (PCOS) have increased risk for metabolic syndrome and cardiovascular disease compared with white women with PCOS [corrected]. Fertil Steril 2014;101:530-5.
16. Rebar R. Evaluation of amenorrhea, anovulation, and abnormal bleeding. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al, editors. Endotext. South Dartmouth (MA): MDText. com, Inc.; 2000.
17. Wijeyaratne CN, Seneviratne RA, Dahanayake S, Kumarapeli V, Palipane E, Kuruppu N, et al. Phenotype and metabolic profile of South Asian women with polycystic ovary syndrome (PCOS): results of a large database from a specialist Endocrine Clinic. Hum Reprod 2011;26:202-13.
18. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.
19. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update 2003;9: 505-14.
20. Unluhizarci K, Kaltsas G, Kelestimur F. Non polycystic ovary syndrome-related endocrine disorders associated with hirsutism. Eur J Clin Invest 2012;42: 86-94.
21. D'Agostino RB Sr, Pencina MJ, Massaro JM, Coady S. Cardiovascular disease risk assessment: insight from Framingham. Glob Heart 2013; 8:11-23.
22. Panidis D, Tziomalos K, Papadakis E, Chatzis P, Kandaraki EA, Tsourdi EA, et al. Associations of menstrual cycle irregularities with age, obesity and phenotype in patients with polycystic ovary syndrome. Hormones (Athens) 2015;14:431-7.
23. Franks S. Polycystic ovary syndrome in adolescents. Int J Obes (Lond) 2008; 32:1035-41.
24. Bani Mohammad M, Majdi Seghinsara A. Polycystic ovary syndrome (PCOS), diagnostic criteria, and AMH. Asian Pac J Cancer Prev 2017;18:17-21.
25. Kuiri-Hanninen T, Kallio S, Seuri R, Tyrvainen E, Liakka A, Tapanainen J, et al. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. J Clin Endocrinol Metab 2011;96: 3432–9.
26. Carmina E, Campagna AM, Lobo RA. A 20-year follow-up of young women with polycystic ovary

syndrome. Obstet Gynecol 2012;119:263-9.
27. Forslund M, Landin Wilhelmsen K, Schmidt J, Brannstrom M, Trimpou P, Dahlgren E. Higher menopausal age but no differences in parity in women with polycystic ovary syndrome compared with controls. Acta Obstet Gynecol Scand 2019;98:320-6.
28. Luque-Ramirez M, Alpanes M, Sanchon R, Fernandez-Duran E, Ortiz-Flores AE, Escobar-Morreale HF. Refferal bias in female functional hyperandrogenism and polycystic ovary syndrome. Eur J Endocrinol 2015;173:603-10.
29. Handelsman DJ, Sikaris K, Ly LP. Estimating age-specific trends in circulating testosterone and sex hormone-binding globulin in males and females across the lifespan. Ann Clin Biochem 2016;53:377-84.
30. Moran C, Knochenhauer E, Boots LR, Azziz R. Adrenal androgen excess in hyperandrogenism: relation to age and body mass. Fertil Steril 1999;71:671-4.
31. Liang SJ, Hsu CS, Tzeng CR, Chen CH, Hsu MI. Clinical and biochemical presentation of polycystic ovary syndrome in women between the ages of 20 and 40. Hum Reprod 2011;26:3443-9.
32. Munzker J, Hofer D, Trummer C, Ulbing M, Harger A, Pieber T, et al. Testosterone to dihydrotestosterone ratio as a new biomarker for an adverse metabolic phenotype in the polycystic ovary syndrome. J Clin Endocrinol Metab 2015;100:653-60.
33. Semmens J, Rouse I, Beilin LJ, Masarei JR. Relationship of plasma HDL cholesterol to testosterone, estradiol and sex hormone binding flobulin levels in men and women. Metabolism 1983;32:428-32.
34. Haffner SM, Kushwaha RS, Foster DM, Applebaum-Bowden D, Hazzard WR. Studies on the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. Metabolism 1983;32: 413-20.
35. Sorva R, Kuusi T, Dunkel L, Taskinen MR. Effects of endogenous sex steroids on serum lipoproteins and postheparin plasma lipolytic enzymes. J Clin Endocrinol Metab 1988;66:408-13.
36. Liou TH, Yang JH, Hsieh CH, Lee CY, Hsu CS, Hsu MI. Clinical and biochemical presentations of polycystic ovary syndrome among obese and nonobese women. Fertil Steril 2009;92:1960-5.
37. Panidis D, Tziomalos K, Macut D, Delkos D, Betsas G, Misichronis G, et al. Cross-sectional analysis of the effects of age on the hormonal, metabolic, and ultrasonographic features and the prevalence of the different phenotypes of polycystic ovary syndrome. Fertil Steril 2012;97:494-500.
38. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640-5.
39. Wild RA, Grubb B, Hartz A, Van Nort JJ, Bachman W, Bartholomew M. Clinical signs of androgen excess as risk factors for coronary artery disease. Fertil Steril 1990;54:255-9.
40. Tzeng CR, Chang YC, Chang YC, Wang CW, Chen CH, Hsu MI. Cluster analysis of cardiovascular and metabolic risk factors in women of reproductive age. Fertil Steril 2014;101:1404-10.