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Serum apelin levels in polycystic ovary syndrome and its relationship with adiposity profile in females.

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ABSTRACT… Objectives: The purpose of the current study was to compare the serum Apelin level in patients with and without polycystic ovarian syndrome to assess this as a diagnostic marker for PCOS. In a case-control study 60 polycystic ovarian syndrome patients diagnosed according to Rotterdam criteria and 20 healthy controls of reproductive age group were recruited. **Study Design:** Case Control study. **Setting:** Jinnah Postgraduate Medical Center in Collaboration with Aga Khan University, Karachi. **Period:** August 2017 and February 2019. **Material & Methods:** Blood samples were collected after 12 hours fasting and was used to test serum Apelin, insulin, cortisol and fasting blood glucose levels. **Results:** Seventy percent PCO subjects had irregular menstrual cycle and 68% suffered from hirsutism; while all PCO subjects had more than 10 antral follicle seen on ultrasound in either one or both ovaries in comparison of 15% controls ($p<0.05$). Higher Apelin and Testosterone levels in PCO group was reported when compared with controls ($p<0.05$) across all BMI categories. Apelin showed a positive correlation with number of ovarian cysts ($r=0.429$; $p=0.000$); irregular menstrual cyle ($r=0.276$;$p=0.13$); blood glucose level ($r=0.270$; $p=0.015$) and BMI ($r=0.229$; $p=0.14$). Apelin remained independently associated with the risk of PCOS ($p=0.020$) and elevated testosterone levels ($p=0.030$). **Conclusion:** Serum Apelin showed a strong association with irregular menstruation, hirsutism, ovarian cysts, testosterone and deranged blood glucose levels. Therefore, Apelin appears as a potential source for several risk factor in PCOS women of all BMI types.

Key words: Apelin, Hirsutism, Irregular Menstrual, Obesity, PCOS.

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INTRODUCTION

Polycystic ovary syndrome is the most frequent endocrine ailment in reproductive age group, its frequency is about 6–8% in the reproductive period.[1] Worldwide the overall commonness and phenotypic highlights of polycystic ovary disorder (PCOS) is the most widely acknowledged endocrine issue among women of reproductive age.[2] This difference in prevalence of PCOS around the globe is mainly due to different diagnostic criteria's and because of cultural antithetic groups. Therefore, to overcome this major diagnostic variation differences there is a need to go for ethnic specific guidelines for PCOS.[3] PCOS is a set of symptoms represented and described by irregular menstrual cycle, sterility, androgen excess and insulin resistance.[4] PCOS has long lasting hazards such as cardiovascular illnesses, Type-2 DM, dyslipidemia and endometrial carcinoma.[5]

Apelin a newly discovered adipo-cytokine is a cell signaling protein secreted by fatty tissue. apelin (APLN) is a peptide, which is programmed by the APLN gene (endogenic ligand for the G-protein-linked APJ receptor).[6] Studies have shown higher apelin levels in PCOS patients with a strong correlation with BMI and insulin resistance.[7] Therefore, we hypothesize that apelin levels might be increased in PCOS subjects irrespective of their BMI status. This study aims to evaluate and compare serum apelin levels in females with PCOS and controls in order to assess Apelin as a diagnostic marker for PCOS.

MATERIAL & METHODS

This case control study was conducted at BMSI-Jinnah postgraduate medical center in collaboration with Aga Khan University, Karachi between August 2017 and February 2019. Ethical approval was obtained from the institutional review committee (NO.F.2-81-IRB/2017-GENL/418/JPMC). A sample size of 80 women was calculated to achieve a power of 90 and an alpha of 5%, with a prevalence of PCOS as 15% in local population.[8] Eighty-five subjects agreed to consent and participate in this study. The inclusion criteria was females of reproductive age group diagnosed as PCOS per Rotterdam criteria. Rotterdam criteria for PCOS diagnosis states that a women may present with any 2 out of 3 conditions: Anovulation; hirsutism or less commonly male pattern alopecia or raised free testosterone; or polycystic ovaries on ultrasound (when 10 small antral follicles are seen in each ovary).[4] Subjects were classified as normal controls if they had no symptom of PCOS as described above. Study participants were selected via convenient sample technique and divided into two groups, Group A: PCO group included individuals with diagnosed polycystic phenotypes n=60 and Group B: Control group included individuals without any PCOS phenotypes n=20.

Subjects with any systemic diseases like atherosclerosis, diabetes mellitus, hypertension and any other reproductive disorders such as congenital adrenal hyperplasia, androgen secreting tumors, cushing syndrome, thyroid dysfunction and hyper prolactinaemia were excluded from this study. After obtaining written and informed consent from the subjects, their biophysical parameters were measured. Ten milliliters of blood was collected after overnight fasting of 10-12 hours and serum was stored at -80°Celsius. Serum samples were used to measure Insulin (cat IN374S by Calbiotech), Cortisol (cat DCMOO1-12) and human Apelin (cat 90291) by enzyme linked immune sorbent assay.

SPSS version 21.0 was used for data entry and exploration. A descriptive statistical analysis of continuous variables was performed. Data on continuous variables i.e. biophysical and biochemical parameters were presented as Mean ± standard deviation (SD) or ± standard error of mean (SEM). Student t-test for continuous/ quantitative variables, chi square or Fischer exact test for categorical variables and Pearson coefficient of correlation (r) was applied. Multiple regression analysis was performed to identify the effect of Apelin on PCOS. In all statistical analysis only p-value < 0.05 was considered significant.

RESULT

The groups were age and weight matched therefore no significant difference was observed ($p>0.05$). However, fasting blood glucose levels in PCOS group was significantly raised as compared to the control group (Table-I). Seventy percent PCOS subjects had irregular menstrual cycle and 68% suffered from hirsutism; while all PCOS subjects had more than 10 antral follicle seen on ultrasound in either one or both ovaries in comparison of 15% controls ($p<0.05$) (Table-I). Higher Apelin and testosterone levels in PCOS group was reported when compared with controls ($p<0.05$), whereas all other markers showed no difference amongst the groups (Table-II). When we stratified the subjects according the BMI category, higher Apelin levels were seen in subjects with PCOS across all BMI categories. Furthermore, Apelin levels showed a positive correlation with number of ovarian cysts ($r=0.429$; $p=0.000$); irregular menstrual cyle ($r=0.276$;$p=0.13$); blood glucose level ($r=0.270$; $p=0.015$) and BMI ($r=0.229$; $p=0.14$). When we applied multiple regressions to adjust for the effect of BMI on Apelin levels, Apelin remained independently associated with the risk of PCOS ($p=0.020$).

DISCUSSION

Polycystic ovary syndrome (PCOS) is considered as a multifaceted syndrome that causes major health problems such as menstrual irregularities, infertility, excess hair, acne, obesity etc.[9] The true mechanism of the syndrome still is not fully understood, but literature hints for a genetic link.[10] In this current study significantly higher level of serum apelin was found in PCOS than normal controls ($p<0.05$).

Variable		Control	Polycystic Ovarian Disease	P-Value
		(n=20)	(n=60)	
Age (Year)		28.80±5.87	26.30±6.16	0.738
Weight (Kg)		63.10±9.18	63.56±13.12	0.115
BMI (Kg/m^2)		26.36±3.65	26.53±4.85	0.122
Fasting Blood Glucose (mg/dl)		85.75±4.98	113.63±35.45	0.001
		Absolute No. (%)	Absolute No. (%)	
Positive family history of diabetes		08 (40%)	19 (31.66%)	0.827
Physically active (Exercise for 30 minutes 3 times a week)		1 (5%)	4 (6.66%)	0.571
Distribution of Rotterdam criteria of participant				
Menstrual Cycle	Regular	20 (100%)	18 (30%)	0.001
	Irregular	0 (0%)	42 (70%)	0.001
Hirsutism		0 (0%)	41 (68.33%)	0.001
Cysts on Ultrasound (>10 small antral follicles)		3 (15%)	60 (100%)	0.001
Ovary	Right	1 (5%)	18 (30%)	0.001
	Left	2 (10%)	18 (30%)	0.044
	Both	0 (0%)	24 (40%)	0.001

Values expressed as Mean ± SD and absolute number with percentage in parenthesis. Comparison between groups was made by Student Test and Chi square test. *Statistically significant as compared to controls p<0.05. **Statistically significant as compared to compared p<0.01

Table-I. Biophysical Data of the Study Cohort.

Variable	Control	Polycystic Ovarian Disease	P-Value
	(n=20)	(n=60)	
FSH (IU/L)	6.90 ±1.37	6.43 ± 34.45	0.114
LH (IU/L)	5.72 ±1.41	5.26 ± 1.12	0.837
Prolactin (ng/ml)	20.53 ±5.83	22.46 ± 1.28	0.722
Estradiol (pg/ml)	178.17 ± 29.74	183.30 ± 7.04	0.765
Testosterone (pg/ml)	0.36 ±0.19	0.72 ± 0.27	0.046
Insulin (uIU/ml)	6.59 ±11.18	8.58 ± 1.63	0.659
Apelin (ng/ml)	175.20 ± 23.87	1827.46 ± 197.28	0.000
Cortisol (ng/ml)	242.58 ± 20.05	233.3 ± 12.86	0.806

Values expressed as Mean ± SD and absolute number with percentage in parenthesis. Comparison between groups were made by Student Test and Chi square test.

Table-II. Biochemical Data of the study Cohort.

Similar results was reported by another study, where elevated levels of Apelin in PCOS were recorded than healthy women.[11] Furthermore, when we categorized the study subjects according to BMI; higher apelin levels were seen in subjects with PCO across all BMI categories, suggesting that apelin has a direct effect on ovarian stimulation of follicles. Similar findings was reported by another control study that stated that apelin levels remained the same in all BMI group of PCOS patients.[12] This study reports higher fasting blood glucose level in PCOS patients than healthy females. This finding is supported by studies conducted by Xianchang et al and Olszanecka-Glinianowicz *et al* who theorized that in PCOS patient blood glucose level, insulin resistance and BMI are positively affected by level of serum Apelin.[13,14] Another study supported this idea by showing a positive relationship between the levels of glucose with total cholesterol (p<0.01), triglycerides (p<0.05), LDL-C (p<0.01). There are other studies that reported that blood glucose level, insulin resistance and BMI are positively affected by levels of serum Apelin which may be

one of the cause of infertility in PCOS females as suggested in our current study.[15,16]

Another important aspect of this study was that 70% PCO subjects had irregular menstrual cycle and 68% suffered from hirsutism. Also all PCO subjects had more than 10 antral follicle seen on ultrasound in either one or both ovaries in comparison of 15% controls ($p<0.05$). One study reported the presence of apelin receptors in human ovarian cells, follicular fluid and granulosa cells in PCOS subjects than that observed in control patients.[17] They postulated that apelin is involved in insulin like growth factor 1 (IGF1) induced steroid biosynthesis which ultimately causes increase formation of ovrian cyst in PCOS and confirm APELIN level strong association with ovarian cyst number. Additionally, higher Apelin and Testosterone levels in PCO group was reported when compared with controls ($p<0.05$), whereas all other markers showed no difference amongst the groups. In another study, PCOS patients showed significantly higher levels of Testosterone compared to healthy controls suggesting a strong link between a high testosterone and adverse metabolic phenotype in PCOS patients.[18]

Besides these promising findings, some of the limitations of this study was its sample size and inability to perform invasive investigation such as ovarian fluid collection to emasure the apelin level. Nevertheless, this study has identified apelin as a possible diagnostic and prognostic biomarker for PCOS patients but further investigations are needed before apelin can be used as treatment for PCOS.

CONCLUSION

Serum Apelin showed a strong association with irregular menstruation, hirsutism, ovarian cysts, testosterone and deranged blood glucose levels. Therefore, Apelin appears as a potential source for several risk factor in PCOS women of all BMI types.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Ashba Allahwasaya	Did data collection and manuscript writing. All authors did review and final approval of manuscript.	
3	Nargis Anjum	Manuscript writing, All authors did review and final approval of manuscript.	
4	Syeda Sadia Fatima	Conceived, designed and did statistical analysis & editing of manuscript. All authors did review and final approval of manuscript.	