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Increased Prolidase Level and Altered Hormonal Profile in Women with Poly Cystic Ovarian Syndrome

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Abstract—*Extracellular matrix is composed of a mixture of various growth factors, macro and micronutrients such as collagens, proteoglycans and glycoproteins. Some of these factors helps in signal transduction and produces signals for cellular growth, differentiation and tissue morphogenesis. Follicle growth in ovaries includes is a stepwise process which results because of tissue remodeling which itself is a consequence of change in the extracellular matrix i.e. follicle growth occur due to stepwise changes in the theca and granulosa cell composition in the developing follicle with respect to time and hormonal level. Therefore, any change in extracellular matrix may leads to abnormal hormonal secretion and may cause disorders Prolidase, a manganese dependent cytosolic matrix metalloproteinase plays an important role in the cell growth and recycling of proline for collagen biosynthesis. It can be considered a key regulator of various pathological processses such as inflammatory response in various diseases, in tissue remodelling also in some cancers and cardiovascular diseases. As it can remodel the tissue matrix, it may play some role in the development of ovarian follicle and also in pathophysiology of poly cystic ovary syndrome. Hence, we aimed to compare the activity of prolidase and hormonal profile in women with PCOS and their controls. PCOS group have significantly higher LH/FSH ratio, prolactin and testosterone than their control group. Significantly higher levels of plasma prolidase were observed in PCOS group in comparison to their control group thereby confirming the role of prolidase in the pathophysiology of PCOS. However, the exact mechanism that prolidase plays needs to be explored.*

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is the one of the most common endocrine-pathological syndrome affecting women of reproductive age, manifested with a variety of clinical signs**[1]**. Because till date its etiology is not defined, so it is difficult to understand its exact pathophysiology. Among number of criterias available to diagnose it, the most accepted criteria is Rotterderm criteria, according to this the polycystic ovary syndrome is diagnosed if a woman has two out of three sets of conditions[2].

- The first is increased levels of male hormone. Acne, excess body hair growth or accelerated loss of hair from the scalp are the result of excess male hormone.
- The second is anovulation, i.e. lack of regular ovulation. This results in infrequent irregular menstrual periods.
- The third is polycystic ovaries.

As PCOS is a syndrome, it is manifested by more than one type of clinical signs and symptoms. It include various physiological, metabolic, reproductive and obestric dysfunction. Physiological, reproductive dysfunction includes lack of periods (amenorrhea), chronic or oligoanovulation, hyperandrogenism and infertility[3].Metabolic dysfunction includes insulin resistance, dyslipidemia, type 2 diabetes and impaired glucose tolerance[4]**.**

Extracellular matrix is composed of a mixture of various growth factors, macro and micronutrients such as collagens, proteoglycans and glycoproteins. These nutrients provide a microenvironment for the surrounding cells for their growth, to differentiate into other cell types. Some of these factors helps in signal transduction and produces signals for cellular growth, differentiation and tissue morphogenesis. Follicle growth in ovaries results because of tissue remodeling which itself is a consequence of change in the extracellular matrix i.e. follicle growth occur due to stepwise changes in the theca and granulosa cell composition in the developing follicle with respect to time and hormonal level. Therefore, any change in extracellular matrix may leads to abnormal hormonal secretion and may cause disorders[5]**.**

Extracellular matrix also includes a group of extracellular proteolytic enzymes collectively named as matrix-mettaloproteinases (MMPs) which cleaves protein substrates including structural proteins of extracellular matrix. These MMPs can be of membrane bound or soluble form that can degrades collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan[6]**.** Under normal physiological condition

MMPS expression is very low and homeostasis is maintained[7]. Their level is regulated by various hormones, growth factors and cytokines. Along with these regulating factors, there are some endogenous MMP inhibitors (MMPIs) and tissue inhibitors of MMPs (TIMPs) which further controls their expression very strictly. Pathological conditions occur when there is an imbalance between activity of MMPs and TIMPs. Mainly the cells of connective tissues and some pro-inflammatory cells such as fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes secretes matrix metalloproteinases. Initially these enzymes are in the form of zymogens which became activated after processing by other proteolytical enzymes like serine proteases, plasmins and furins. In normal condition their activity is regulated at various levels whereas in pathological conditions equilibrium is shifted in either direction resulting in altered collagen metabolism [8].

Prolidase accepted as MMP on biochemical level is a cytosolic exopeptidase depend on manganese (Mn) catalyses the final step of collagen metabolism (degradation) by removing imidodipeptides containing C-terminal proline or hydroxyproline and results in matrix remodeling and cell growth[9,10].

Prior studies have suggested that MMPs may be involved in pathogenesis of PCOS via regulating ovarian tissue remodeling. However no such study has been conducted on the role of prolidase with in pathophysiology of PCOS in India. Therefore the present study aims to assess the activity of Prolidase in PCOS patients in comparison to their controls.

2. MATERIALS AND METHODS:

Subjects:

Study was conducted in the women of age between 15-35 years. Forty PCOS and sixty two healthy non hyperandrogenic women were studied. Women of the control group were healthy volunteers with normal ovulatory cycles, with no signs of hyperandrogenism and normal sonographic appearance of the ovaries. None of the women who studied had galactorrhea or any additional systemic disease. Informed consent for participation of all women under study was obtained and the study was approved by Institutional Ethical Committee.

A questionnaire was designed and applied face to face to gather data on the identification of the patients as well as their anthropometric characteristics, pubertal, menstrual, sexual and reproductive history.

Samples:

Plasma was used to access the prolidase activity. Blood samples were collected from Post Graduate Institute Of Medical Sciences (PGIMS), Rohtak. 2 ml blood was withdrawn by venipuncture and collected in heparinised vacutainer. Blood samples were immediately transferred to ice bucket. Plasma was separated by centrifuging the whole blood at 3000 rpm for 5 minutes at 4 °C on the same day of collection.

Prolidase activity Assay:

Blood plasma was incubated with Mn^{+2} for the activation of plasma prolidase. This was done by mixing 100µl of 0.05mol/l Tris HCl($_p$H-7.8) and 100 µl of 20mmol/l $MnCl_2$ in 100µl of plasma followed by incubation at 37^{o}C for one hour. Then 100 µl of incubated solution was mixed with 100µl of 94mmol/l glycine-proline solution and this mixture was again incubated for one hour at 37^{o}C. After 1 hour, 1 ml of 0.45mol/l Trichloroacetic acid was added to stop further reaction. To 0.5 ml of supernatant, 2 ml of 1:1 mixture of glacial acetic acid: chinard reagent was added following incubation at 90 ^{o}C for 10 min. Absorbance was taken at 515 nm and prolidase activity was calculated by using proline standard[11,12].

Measurement of hormonal profile:

Luteinizing hormone, Follicle Stimualtng hormone, Prolactin, and testosterone were measured by manufacterer's protocol supplied in kits.

3. RESULTS AND DISCUSSION:

Anthropometric parameters included in study are age, height, weight, BMI and hip/waist ratio (Table - 1). No significant difference was observed between age, height of PCOS patients and their controls. But there was significant difference in the BMI of the two groups. PCOS group has significantly higher BMI than control group. Higher BMI is related to metabolic changes that can lead to anovulation. These changes include peripheral aromatization of estrogens, low levels of sex hormone-binding globulin resulting in increased levels of free estradiol and testosterone, and higher insulin levels that can leads to increased ovarian production of androgens[13].

Table 1: Anthropometric data of PCOS and control patients

Name of parameter	PCOS subjects	Control
Age	24.13±6.26*	24.54 ± 4.23
Weight	60.88±13.08**	52.78 ± 3.67
BMI	23.77±4.80***	22.10 ±.095
Hip/Waist ratio	37.40±4.55***	34.78 ± 4.98

* non significant
** significant
*** highly significant

One of the major cause of PCOS is altered hormonal level. Increased androgen (testosterone) production the key manifestation of PCOS results in excess facial and body hair (hirsutism), acne and male-pattern baldness in women.. Higher androgen production is related with the higher level of LH. LH supports the theca cells and responsible for the development of corpus luteum. These theca cells produce excess androgen in the ovary. In our study we also found higher levels of testosterone, LH and prolactin (table - 2) in women having

PCOS than their controls however no significant difference was found in FSH level between the two groups.

Table 2: Hormonal and Enzymatic profile of PCOS and Control group

Biochemical Parameters	PCOS mean± SD, n=40	CONTROL Mean± SD, n=60
Prolidase level (IU/ml)	1200± 52.82,	900±51.56
FSH(mIU/ml)	6.20±.90	6.50± 1.03
LH(mIU/ml)	5.70± 2.28	4.50± 1.70
PRL(ng/ml)	22.59± 0.25	15.17± 0.18
Testosterone(ng/ml)	0.079± 0.020	0.38±0.06

MMPs are a multigene family of more than 25 free or membrane bound extracellular enzyme that degrade ECM. They target nearly all the components of ECM such as growth factors, growth factor binding proteins, cell adhesion moleclues, other proteases, cell surface receptors, released protein signalling molecules, basically majority of the protein content of extracellular matrix, this makes them potent regulator of many biological processes. There are a handful of studies (table-3) conducted on PCOS and matrix metalloproteinase. As they degrades extracellular matrix, thereby facilitating remodelling. Prolidase is one of the matrix metalloproteinase involved in recycling of proline in collagen metabolism and tissue remodeling.

Table 3: Studies done on PCOS and MMPs

Year	Author	Population studied/ country	Sample used	MMP/ TIMP studied	Resuts
2001	Shalev et al.	Europe	Follicular fluid	MMP 2 and 9	Higher levels of MMP-2, MMP-9 in PCOS group
2003	Lahav-Baratz et al.	Israel	Follicular fluid	MMP 2 and 9	Similar levels of MMP-2 and MMP-9 in PCOS group
2006	Lewandowski et al.	British	Serum	MMP 2 and 9	Higher levels of MMP-2, MMP-9 in PCOS group
2008	Liu et al.	China	Serum	MMP 9/TIMP 1	Higher MMP-9, MMP-9/TIMP-1ratio in PCOS group
2010	Baka et al.	Czech republic	Serum/ follicular fluid	MMP 2 and 9/ TIMP 2 and 1	Higher levels of MMP-2 and MMP-9 TIMP-2 and TIMP-1 in PCOS group
2010	Nese Hilali et al	Turkey	Serum/plasma	Prolidase	Increased prolidase activity in PCOS group
2011	Gomez et al	Brazil	Plasma	MMP 2 and 9/ TIMP 2 and 1	Higher MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios in PCOS group
2015	Akcali et al	Turkey	Saliva	MMP8 /TIMP 1	Higher MMP8 /TIMP1 in PCOS group

In 2003 lahav –Baratz observed an increased concentration of MMP-2 and MMP-9 in the follicular fluid of the women having PCOS when compared to healthy women[14]. Similar results were found in follicular fluid as well as in serum samples of PCOS patients by Lewandowski et.al[15]. In 2004, Lie et.al., found increased ratio of MMP-9/TIMP-1 along with high concentration of MMP-9 in the serum samples of women with PCOS when compared with healthy controls. All the studies about the assessment of MMPs and TIMPs in PCOS clearly indicate that increased gelatinolytic activity is associated with PCOS[15,16,17,18]. These studies provides a clear insight that imbalance of MMPs concentration is related to pathopysiology of PCOS. Prolidase is involved in the metabolism of collagen hence its deficiency involves a number of metabolic and pathological disorders.

So we decided to assess prolidase activity in plasma samples of women with PCOS. In India, the present study is the first to assess plasma prolidase activity in the group of women with PCOS. Women with PCOS have increased cardiovascular risk such as ‘early onset’ atherosclerosis and cardiac dysfunction independent of weight[4,19] Moreover measurement of circulating levels of ECM turnover biomarkers such as the MMPs and the TIMPs have long been used in the evaluation of atherosclerosis[20]. It has been found that prolidase activity is altered in various diseases like benign prostatic hyperplasiasystemic sclerosis, liver fibrosis, pulmonary tuberculosis, acute hemorrhagic stroke and a number of cancer [21,22,23,24,25]. In our study we found significantly increased level of prolidase in the plasma sample of PCOS group (1200 IU ±52.82) than the control group (900 IU ±51.56).

Hence we can conclude that prolidase activity is associated with PCOS development and may be causing ovarian tissue remodeling which can ultimately lead to ovarian cancer. But

this was a small study and to the best of our knowledge no other study in India had been previously done. The exact origin of prolidase activity and the extent of the ovarian contribution of prolidase is not clearly defined so it needs to be evaluated on a larger scale in future.

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