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Research Article

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Role of Cystic Fluid Growth Factors and Inflammatory Mediators in Pathogenesis of Polycystic Ovary Syndrome

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Abstract

Objective: *Present study was carried out to investigate whether there is any localized growth factor/s involved and mediated by inflammatory cytokines through measuring their level in fluid aspirated from ovarian cyst.*

Study Design: *Data was collected from 62 infertile female patients having PCOS and without PCOS. The cystic and follicular fluid obtained from consented patients was used in estimating inflammatory and growth factors. Further, these values were analyzed and interpreted with respect to number, size, length and depth of cysts.*

Results: *Indicates that there is a low level of inflammatory reaction centralized to IL-18 triggering the release of MMP-2 to degrade the extracellular matrix followed by release of ANG-1, VEGF, IGF-1 and EGF to maintain the growth of ovarian cysts. Our findings support the prominent role of IL-18, MMP-2 and ANG-1 in pathogenesis of severe PCOS.*

Conclusion: *In conclusion, the levels of inflammatory mediators in follicular fluid are significantly higher in women having PCOS than compared to cystic fluid of Non-PCOS patients. Despite the promising results of present study we recommend the readers to consider these results as preliminary. Further larger cohort studies are recommended by including more number of heterogeneous population, for extended period of time and more number of cytokines and growth factors with higher accuracy to understand how local inflammatory and growth factors are inter-related in development of ovarian cysts in PCOS patients.*

Keywords: Proliferation; Immunomodulation; Inflammation; Angiogenesis; Cytokines; Growth Factors; Polycystic Ovary Syndrome (PCOS)

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common chronic complex endocrine disorders commonly seen in reproductive age group women. It has been proven that chronic low grade inflammatory process is involved in pathogenesis of ovarian cyst [1]. This chronic low grade inflammatory process is mediated by increased serum white cell count due to increase in number of neutrophils [2]. The increase in inflammatory cellular count is followed by secretion of increased serum proinflammatory cytokines tumor necrosis factor alpha (TNF-alpha), TNF soluble receptors, IL-6, nitric oxide, C-reactive protein (CRP), complement C3, high-sensitivity C-reactive protein and free fatty acids [3-6]. These inflammatory mediators are reported to overexpress nerve growth factor, NFkappaB, inhibitory kappaB, p65, migration inhibitor fact, monocyte chemo attractant protein and macrophage inflammatory protein -1 alpha [1, 7-9]. The outcome of these mechanisms is proposed to be associated with multiple short, medium and long term consequences such as endometrial cancer, endometrial hyperplasia, hyperandrogenism, impaired glucose tolerance, diabetes, hypertension, myocardial infarction and vascular lesions [7,10,11]. Epithelial cells are known to secrete fluid and electrolytes in female reproductive tract required for the survival of the tissues.

The secretion of fluid and electrolytes in ovarian follicles is regulated by blood-follicle barrier majorly constituted by basement membrane of the developing follicles [12]. It is also being proven that the volume and composition of the fluid secreted is regulated by the proteins, Tran's membrane regulators and ion channels [1]. The fluid and electrolytes secreted are involved in defensive mechanism from infection by trapping pathogens and mechanically remove them via mucociliary clearance while inhibiting their growth [13]. It is also known that the regulated volume and composition of the reproductive tract also play an important role in sperm capacitation and implantation [14]. The fluid filled follicles in ovary called cysts

are divided into four main type's benign non-neoplastic, benign neoplastic, low malignant potential and malignant based on their histopathological characteristics [15]. The change in histologic composition of the follicles such as number of follicles, thickness of the capsule, size of cyst change the fluid volume, composition and complexity of follicles [16].

The pathogenesis and pathophysiology of PCOS point towards the involvement of regrowth of cyst after surgical resection. However, the successful treatment of PCOS remains distant dream due to the fact that all studies until now are correlating pathogenesis of PCOS to obesity and insulin resistance. Review of literature clearly shows limited knowledge on involvement of local growth factors despite of the fact that cysts in ovary remain localized with respect to their growth and presence. Therefore, it is very important to investigate the role of these factors in localized tissues of ovary. The best way of studying them is by analyzing the presence and levels of these growth factors in cystic fluid obtained from the cysts of the patients who has undergone surgical procedure. It is important to underline here that most of the studies have correlated systemic low grade inflammatory changes to the presence of cyst in ovaries. However, localized inflammatory process activated growth factors and their correlation to pathogenesis of cyst in ovary is not been studied.

Therefore, present study was carried out to investigate whether there is any localized growth factor/s involved and mediated by inflammatory cytokines through measuring their level in fluid aspirated from ovarian cyst. Outcome of present study will assist in understanding the role and their extent of role in pathogenesis of PCOS which can be used in developing biomarker/s for diagnosis, developing therapeutic strategy, monitoring safety and efficacy of therapy. PCOS in female is commonly associated with multiple metabolic abnormalities however; studies in Oman are still limited. The aim of this study is to investigate involvement of localized mechanism in pathogenesis of PCOS through estimation of level of TNF-α, IL-6, EGF, IGF-1, VEGF, ANG1, MMP-2, IL-18 and C3 and their correlation to characteristics of ovarian cysts.

Materials and Methods

Study population

It was a pilot study carried out in randomly selected 62 patients of which 36 were diagnosed with PCOS according to Rottandam Criteria with good health with normal puberty and sexual developments [17]. Other 26 patients were Non-PCOS considered as control but these patients were undergoing IVF after ovary hyperstimulation (OHS). Study was carried out at Al Bushra Medical Specialty Complex, Muscat among the women undergoing surgical removal of ovarian cysts and IVF respectively. All patients volunteered in the study were asked to submit written consent for specimen collection. Patients with androgen-secreting tumors in ovaries, Cushing's syndrome, recent urinary tract infection, inflammation due to other reasons, hyperprolactinemia, or thyroid disorders and taking NSAIDs or steroidal therapy were excluded from the study.

Cystic fluid collection

The study was carried out in ovarian cystic fluid collected from women who underwent laparoscopic for polycystic ovary and intrafollicular fluid of OHS patients undergoing IVF. At least two ovarian follicles removed from each patient were aspirated separately and centrifuged to remove cell debris, and all supplements were stored at -20oC until use [18]. The concentration of TNF-α, IL-6, IL-18, EGF, IGF-1, VEGF, C3, angiopoeitin-1 and MMP-2 in each sample were measured by ELISA kits as recommended by manufacturer [19]. The level of intra-follicular factors in PCOS patients were compared with intra-follicular level in patients undergoing IVF as control. Control group of patients are those having no past and present history of PCOS and matching with respect to medical condition.

Principle of the ELISA Assay

ELISA kits purchased from Wuhan Fine Biotech Co., Ltd., China was based on sandwich enzyme-linked immune-sorbent assay technology [20]. Antibody was pre-coated onto 96-well plates and the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP- Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a micro plate reader, and then the concentration of each cytokine was calculated by using Curve Expert 1.4 software.

Follicular Characteristics

The ultrasonography report of the patient collected from case file and the number of follicles, follicular size, length, depth and pattern of distribution such as central, peripheral or mixed were recorded in data record sheet. The number of follicles were stratified as few if <5; moderate 5-10 and multiples>10 [20]. Variation in the level of intra-follicular factors with respect to follicular characteristics were analyzed to gauze their relationship and level of severity.

Statistical analysis

The SPSS statistical package for windows ver. 23.0 (SPSS Inc., Chicago, IL- USA) was used for all data analysis. Students 't' test was used for descriptive statistical analysis. Pearson correlation coefficient test was used to rank difference variables against each other in linear correlation whether positive or negative. Chi-square test was used to compare qualitative variables. ROC curve was used to find out the best cut off value of certain variables and $p<0.05$ considered significant. Multinomial linear regression model was used to study relationship between PCOS characteristics and each growth factor. Dendrogram with average linkage is used to express different levels of relationship between multiple factors and characteristics of cysts.

Results

Comparison of level of cytokines in Control and PCOS follicular fluid

The age of patients with an ovulatory infertility due to PCOS was significantly ($p<0.01$) lower 30.06 ± 4.37 compared to 33.76 ± 5.52 control. The levels of IL-6, IL-18, IGF-1 and C3 were significantly ($p<0.01$) lower than compared to control group of patients. (Table-1)

Table 1: Comparison of level of cytokines in Normal and PCOS follicular fluid

	Type	N	Mean ± Std. Deviation
Age	Control	25	33.76 ± 5.52
	PCOS	36	30.06 ± 4.37**
Number of cysts	PCOS	36	19.33 ± 3.59
Cyst Size	PCOS	35	17.46 ± 1.12
Cyst Length mm	PCOS	36	15.88 ± 5.45
Cyst Depth cm	PCOS	36	6.25 ± 0.44
TNF (pg/mL)	Control	26	5.09 ± 16.2
	PCOS	36	00
IL-6 (pg/mL)	Control	26	804.89 ± 1309.81
	PCOS	36	15.13 ± 34.57***
IL-18 (pg/mL)	Control	26	633.95 ± 312.31
	PCOS	36	373.9 ± 229.82***
IGF-1 (pg/mL)	Control	26	4806.53 ± 5350.83
	PCOS	36	877.27 ± 1451.14***
MMP-2 (pg/mL)	Control	26	3.41 ± 10.53
	PCOS	33	14.28 ± 32.94
VEGF (pg/mL)	Control	26	2594.39 ± 3364.48
	PCOS	36	2468.86 ± 3147.38
EGF (pg/mL)	Control	26	828.77 ± 2747.32
	PCOS	36	80.59 ± 134.12
C3 (pg/mL)	Control	26	1615.25 ± 1367.77
	PCOS	36	842.49 ± 327.89**
ANG (pg/mL)	Control	26	635.47 ± 2156.11
	PCOS	36	707.93 ± 949.36

****The PCOS values are highly significantly ($p<0.001$) varying from control.*
*** The PCOS values are very significantly ($p<0.01$) varying from control.*

Pearson correlation analysis between characteristics of cyst and cytokines

The values with $p<0.01$ is shaded with yellow. Data in table- 2 depicts that MMP-2 has significant positive correlation to length of the cyst whereas IL-6, IL-18, IGF-1 and C3 have negative correlation to number, length, depth and size of cysts. Similarly, TNF-alpha has positive correlation with Angiopoietin-1; IL-6 with EGF; IL-18 with IGF-1, VEGF, EGF and C3; IGF-1 with IL-18, VEGF and C3; VEGF with IL-18, C3 and Angiopoietin-1; EGF with IL-6; C3 with IL-18, VEGF, and IGF-1; Angiopoietin-1 with TNF-alpha and VEGF.

Table 2: Pearson Correlation Analysis of Characteristics of Cysts and Cytokines

pg/mL	Number of cysts	Cyst Size	Cyst Length	Cyst Depth	TNF	IL-6	IL-18	IGF-1	MMP-2	VEGF	EGF	C3	ANG-1
TNF	-0.229	-0.236	-0.211	-0.236	1	-0.036	0.057	0.175	-0.015	-0.009	-0.039	0.066	0.341**
IL-6	-0.407**	-0.236	-0.211	-0.236	1	-0.036	0.057	0.175	-0.015	-0.009	-0.039	0.066	0.341**
IL-18	-0.453**	-0.421**	-0.379**	-0.422**	-0.036	1	0.166	0.164	-0.099	-0.052	0.794**	0.142	-0.160
IGF-1	-0.465**	-0.433**	-0.400**	-0.416**	0.057	0.166	1	0.343**	0.215	0.402**	-0.086	0.395**	0.239
MMP-2	0.086	-0.484**	-0.452**	-0.478**	0.175	0.164	0.343**	1	-0.033	0.332**	0.040	0.823**	0.114
VEGF	-0.004	0.224	0.304*	0.194	-0.015	-0.099	0.215	-0.033	1	0.102	-0.078	-0.118	0.225
EGF	-0.200	-0.024	-0.010	-0.013	-0.009	-0.052	0.402**	0.332**	0.102	1	-0.072	0.315*	0.342**
C3	-0.357**	-0.205	-0.190	-0.207	-0.039	0.794**	-0.086	0.040	-0.078	-0.072	1	-0.062	-0.088
ANG-1	0.010	-0.385**	-0.330**	-0.386**	0.066	0.142	0.395**	0.823**	-0.118	0.315*	-0.062	1	0.027
		0.016	0.130	0.020	0.341**	-0.160	0.239	0.114	0.225	0.342**	-0.088	0.027	1

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

Linear Regression Analysis of Cytokines on Number of Cysts

Table -3 shows that there are two factors i.e. IL-18 and IGF-1 are significantly influencing the number of cysts.

Table 3: Linear Regression Analysis of Cytokines on Number of Cysts

Coefficients[a]				t	Sig.
	Unstandardized Coefficients		Standardized Coefficients		
Unit (pg/mL)	B	Std. Error	Beta		
(Constant)	17.475	2.340		7.469	.000
TNF	-0.149	0.105	-0.162	-1.424	0.161
IL-6	-0.003	0.002	-0.304	-1.563	0.124
IL-18	-0.015	0.005	-0.436	-3.243	0.002
IGF-1	-0.001	0.000	-0.504	-2.640	0.011
MMP-2	0.047	0.042	0.122	1.119	0.269
VEGF	0.001	0.000	0.229	1.881	0.066
EGF	0.000	0.001	0.065	0.342	0.734
C3	0.002	0.002	0.228	1.156	0.253
ANG-1	0.000	0.001	0.076	0.588	0.559

a. Dependent Variable: Number of Cysts

Correlation Matrix of Multiple Factors with Number of Cysts

The plotting of matrix of multiple factors with the number of cysts (Table-4) shows that the Angiopoietin-1 has positive correlation with IGF-1 and MMP-2; C3 has negative correlation with IL-18, MMP-2 and VEGF; EGF has negative correlation with IL-18; Angiopoietin-1 has positive correlation with IGF-1 and MMP-2; C3 has negative correlation with IL-18, MMP-2 and VEGF.

Table 4: Correlation Matrix of Multiple Factors with Category of Cysts

Correlation	TNF (pg/mL)	IL-6 (pg/mL)	IL-18 (pg/mL)	IGF-1 (pg/mL)	MMP-2 (pg/mL)	VEGF (pg/mL)	EGF (pg/mL)	C3 (pg/mL)	ANG-1 (pg/mL)
TNF (pg/mL)	1.000	.	.	.	.	.	.	.	.
IL-6 (pg/mL)	.	1.000	0.110	-0.137	0.136	0.102	0.122	-0.229	-0.246
IL-18 (pg/mL)	.	0.110	1.000	0.004	0.514	0.192	-0.405	-0.351	0.256
IGF -1 (pg/mL)	.	-0.137	0.004	1.000	-0.016	-0.137	-0.128	0.211	0.351
MMP-2 (pg/mL)	.	0.136	0.514	-0.016	1.000	0.118	-0.191	-0.391	0.562
VEGF (pg/mL)	.	0.102	0.192	-0.137	0.118	1.000	0.137	-0.377	0.017
EGF (pg/mL)	.	0.122	-0.405	-0.128	-0.191	0.137	1.000	-0.119	-0.171
C3 (pg/mL)	.	-0.229	-0.351	0.211	-0.391	-0.377	-0.119	1.000	-0.026
ANG-1 (pg/mL)	.	-0.246	0.256	0.351	0.562	0.017	-0.171	-0.026	1.000

a. Only cases for which Follicle category = Multiples are used in the analysis phase.

ROC Curve to calculate area under curve

Figure 1 and Table 5 shows that Angiopoietin-1 (0.878) good predictor for number of cysts and MMP-2 (0.65) is a poor predictor of number of cysts. Whereas others failed to show any level of prediction or diagnosis of number of cysts in PCOS patients.

Table 5: Area under the Curve

Test Result Variable(s)	Area
TNF (pg/mL)	0.423
IL-6 (pg/mL)	0.206
IL-18 (pg/mL)	0.260
IGF-1 (pg/mL)	0.155
MMP-2 (pg/mL)	0.650
VEGF (pg/mL)	0.520
EGF (pg/mL)	0.378
C3 (pg/mL)	0.283
ANG-1 (pg/mL)	0.878
The test result variable(s): TNF (pg/mL), IL-6 (pg/mL), IGF-1 (pg/mL), MMP-2 (pg/mL), EGF (pg/mL), ANG-1 (pg/mL) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.	

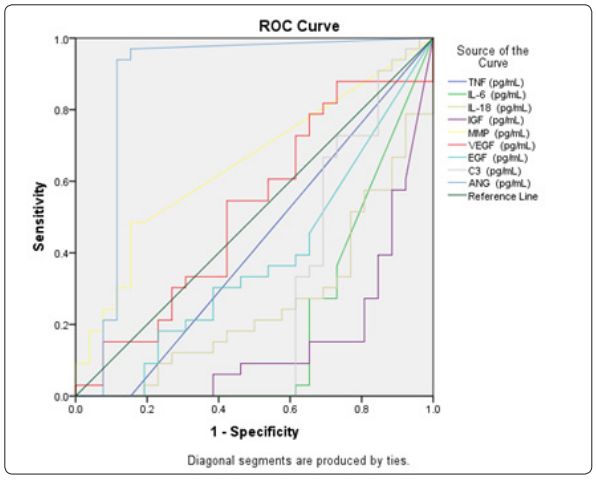

Figure 1: ROC Curve to calculate Area under Curve

Dendrogram using average Linkage

Dendrogram of cytokines and characteristics of cysts clusters derived from average linkage (Figure 2). Factors are divided into five levels of clusters. Group 1 is clustered at first level by including number of cysts, size, length, depth, TNF, MMP-2, and IL-18. The central of all these clusters is IL-18 surrounded by IL-6 and MMP-2. Denrogram clearly shows that IL-18, MMP-2 and TNF-alpha are directly related to numbers, size, length and depth of cysts. IGF-1, VEGF, EGF and Angiopoietin-1 are distantly influencing growth of cysts. Dendrogram clearly depicts that localized PCOS mechanism with respect the size, length, numbers and depth of cysts is centered around inflammatory mechanism mediated by IL-18, TNF-alpha, and MMP-2.

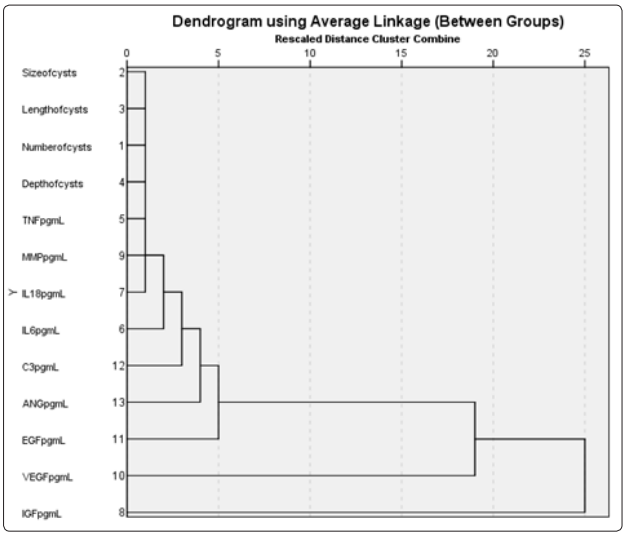

Figure 2: Dendrogram of cytokines and characteristics of cysts clusters derived from average linkage

Discussion

The main aim of the study was attained by including sufficient number of case and controls. Findings of the study shows the role of inflammatory mediators in initiating the inflammatory mechanism and then followed by stimulating growth of cysts. These findings are the most important revelation that there is a localized mechanism mediating inflammatory mediators and growth factors in growth and re-growth of cysts.

Women considered in PCOS group were younger than compared to control group of patients similar to the earlier study [21]. Control group of women considered in this study have undergone ovary hyperstimulation (OHS) for IVF procedure. It is well known that the fluid in follicles is formed by diffusion of blood constituents through blood-follicle membrane barrier and biological active molecules are secreted from associated cells mainly theca cells and granulosa cells [22]. OHS using gonadotropins is believed to induce inflammatory reaction leading to massive extravasation of fluids, inflammatory mediators, enlarging ovary and release of other proteins at varying levels [23]. Inflammatory reaction phase is followed by anti-inflammatory mechanism during maturation of follicles [24]. Whereas, ovarian cysts developed in PCOS patients are the structures with thin granulosa cells [25]. Our results supports above study outcomes as the levels of IL-6, IL-18, IGF-1 and C3 were significantly ($p<0.01$) higher in control group of patients compared to PCOS. These results also supports earlier studies reporting the lower levels of IL-6, VEGF, Angiopoietin-1, CRP, and IGF-1 in line with previous studies [25,26].

The role of follicular fluid in PCOS is recently shown to have inverse correlation with VEGF, TNF-alpha, IL-6 and CRP [26]. There is a positive correlation between VEGF, Angiopoietin-1, IGF-1, C3and IL-18 supporting the hypothesis of their coordinated effect in folliculogenesis, angiogenesis and ovarian vasculature [26,27]. It is very clear from above data that IL-18 and C3 induced inflammatory reaction with respect to numbers, size, length and depth of cysts is reversed by IGF-1, VEGF, MMP-2 and C3 supporting the results of previous review report on inflammatory and anti-inflammatory mechanism [28].

In-vitro studies shown that the IL-1 category of interleukins activated hypothalamus-pituitary- adrenaline axis mediating adrenal steroidogenesis and immune regulatory mechanisms affecting the process of follicle development, fertilization and implantation [29]. IL-18 being a part of IL-1 superfamily it is expected to amplify inflammatory reaction followed by anti-inflammatory mechanism. Our results show a significant reverse correlation between non-liver synthesized IGF-1 and number of cysts in line with a very recent study [30]. It is known to enhance phagocytic mechanism of macrophages for LDL and production of cytokines, chemokines and chemoattractants [31]. The local IGF-1 is known to involve in ovarian dysfunction and folliculogenesis [31].

Interleukin-6 is known to have both inflammatory and anti-inflammatory properties that could be a reason for no significant correlation with other factors [32]. Correlation matrix of multiple factors considered in the study shows that ANG-1 is positively correlated to IL-18, IGF-1 and MMP-2 indicates that the process involves inflammatory reaction followed by anti-inflammatory mechanism mediated by IGF-1, MMP-2 and ANG-1 [25].

MMP is involved in extracellular matrix synthesis, degradation, homeostasis regulation, microenvironment improvement and bioactive molecules regulation [33]. ANG-1 is a part of VEGF expressed in endometrium as well as in ovary has multidimensional activity spurting the proliferation of endothelial cells and neovascularization [23,34]. The effect of these factors is carried forward by IL-6, IL-8, TGF-β1, PPAR-γ and others [29]. Present study outcomes substantiate previous hypothesis on role of MMP and ANG to specific and sensitive predictability on number of cysts. MMPs are zinc-containing peptidases also responsible for tissue growth, expansion and blood vessel development [35]. It is proposed that MMP-2 is involved in separation of the granulosa cells from the theca cells, endometriosis and pathogenesis of endometrial cancer [36,37]. The prominent levels of MMP-2 in present study could be due to involvement of severe category of PCOS patients supporting above studies.

The central role of IL-18 illustrated in our results is well established in proliferative phase of endometrium and endometrial tissue samples supporting its significance in PCOS pathogenesis [38]. This process is followed by release of growth factors that mediate and maintain the growth of cysts in PCOS patients.

This study is the first in its type to analyze levels of inflammatory and growth factors in ovary cystic fluid of PCOS patients and compares with OHS patients. Results of our study illustrated the individual and together role of multiple cytokines and growth factors at different levels of pathogenesis of ovarian cysts. There were many limitations in the study. First the sample size is limited due to less number of patients visiting study center during Holy month of Ramadan. Second the accuracy of the ELISA kits used in the study is 82-97% and the Intra-assay and inter-assay precision is < 8 % and < 10 % respectively. Third, the deep freezer available in our laboratory can maintain the temperature -20oC therefore the study could not be extended beyond two months to include more number of patients. Fourth, study was conducted in only one center therefore it has limited generalizability. Fifth, all PCOS patients are diagnosed with severe PCOS. Last one is that the control group of patients is ovary hyperstimulated patients not normal.

Conclusion

In conclusion, the levels of inflammatory mediators in follicular fluid is significantly higher in women undergone OHS than compared to cystic fluid of PCOS patients. Indicating that there is a low level of inflammatory reaction centralized to IL-18 triggering the release of MMP-2 to degrade the extracellular matrix followed by release of ANG-1, VEGF, IGF-1 and EGF to maintain the growth of ovarian cysts. Our findings support the prominent role of IL-18, MMP-2 and ANG-1 in pathogenesis of severe PCOS. Despite the promising results of present study we recommend the readers to consider these results as preliminary due to wide variation in results. Further larger cohort studies are recommended by including more number of heterogeneous population, for extended period of time and more number of cytokines and growth factors with higher accuracy to understand how local inflammatory and growth factors are inter-related in development of various stages of ovarian cysts in PCOS patients.

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References

1. González F, Rote NS, Minium J, Kirwan JP (2006) Increased activation of nuclear factor kappa B triggers inflammation and insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab 91: 1508-1512.
2. Herlihy AC, Kelly RE, Hogan JL, O'Connor N, Farah N, et al. (2011) Polycystic ovary syndrome and the peripheral blood white cell count. J Obstet Gynaecol 31: 242-244.
3. Yang S, Li Q, Song Y, Tian B, Cheng Q, et al. (2011) Serum complement C3 has a stronger association with insulin resistance than high-sensitivity C-reactive protein in women with polycystic ovary syndrome. Fertil Steril 95: 1749-1753.
4. Rajendran S, Willoughby SR, Chan WP, Liberts EA, Heresztyn T, et al. (2009) Polycystic ovary syndrome is associated with severe platelet and endothelial dysfunction in both obese and lean subjects. Atherosclerosis 204: 509-514.
5. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Kocełak P, Janowska J, Semik-Grabarczyk E (2008) The effect of weight loss on inflammation in obese women with polycystic ovary syndrome. Endokrynol Pol 59: 13-17.
6. González F, Sia CL, Stanczyk FZ, Blair HE, Krupa ME (2012) Hyperandrogenism exerts an anti-inflammatory effect in obese women with polycystic ovary syndrome. Endocrine 42: 726-735.
7. Glintborg D, Andersen M, Richelsen B, Bruun JM (2009) Plasma monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1alpha are increased in patients with polycystic ovary syndrome (PCOS) and associated with adiposity, but unaffected by pioglitazone treatment. Clin Endocrinol (Oxf) 71: 652-658.
8. González F, Rote NS, Minium J, Weaver AL, Kirwan JP (2010) Elevated circulating levels of macrophage migration inhibitory factor in polycystic ovary syndrome. Cytokine 51: 240-244.
9. Wilson JL, Chen W, Dissen GA, Ojeda SR, Cowley MA, et al. (2014) Excess of nerve growth factor in the ovary causes a polycystic ovary-like syndrome in mice, which closely resembles both reproductive and metabolic aspects of the human syndrome. Endocrinology 155: 4494-4506.
10. Puurunen J, Piltonen T, Morin-Papunen L, Perheentupa A, Järvelä I, et al. (2011) Unfavorable hormonal, metabolic, and inflammatory alterations persist after menopause in women with PCOS. J Clin Endocrinol Metab 96: 1827-1834.
11. González F, Sia CL, Shepard MK, Rote NS, Minium J (2012) Inflammation in response to glucose ingestion is independent of excess abdominal adiposity in normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab 97: 4071-4079.
12. Siu MK, Cheng CY (2012) The blood-follicle barrier (BFB) in disease and in ovarian function. Adv Exp Med Biol 763: 186-192.
13. Wine JJ, Joo NS, Choi JY, Cho HJ, Krouse ME, et al. (2011) Measurement of fluid secretion from intact airway submucosal glands. Methods Mol Biol 742: 93-112.
14. Chan HC, He Q, Ajonuma LC, Wang XF (2007) Epithelial ion channels in the regulation of female reproductive tract fluid microenvironment: implications in fertility and infertility. Sheng Li Xue Bao 59: 495-504.
15. Greenebaum E (1996) Aspirating Non-neoplastic Ovarian Cysts: Rationale, Technique, and Controversy. Laboratory Medicine 27: 462-467.
16. Romualdi D, Franco Zannoni G, Lanzone A, Selvaggi L, Tagliaferri V, et al (2011) Follicular loss in endoscopic surgery for ovarian endometriosis: quantitative and qualitative observations. Fertil Steril 96: 374-378.
17. Rotterdam ESHRE/ASRM–Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81: 19-25.
18. Kim CH, Ahn JW, You RM, Kim SH, Chae HD, et al. (2011) Pioglitazone treatment decreases follicular fluid levels of tumor necrosis factor-α and interleukin-6 in patients with polycystic ovary syndrome. Clin Exp Reprod Med 38: 98-102.
19. Wuhan Fine Biotech Co., Ltd. C6-323 Biolake, No. 666 Gaoxin AVE. East Lake High-tech Development Zone, Wuhan, China 2017.
20. Pawelczak M, Rosenthal J, Milla S, Liu YH, Shah B (2014) Evaluation of the pro-inflammatory cytokine tumor necrosis factor-α in adolescents with polycystic ovary syndrome. J Pediatr Adolesc Gynecol 27: 356-359.
21. Shafiee MN, Seedhouse C, Mongan N, Chapman C, Deen S, et al. (2016) Up-regulation of genes involved in the insulin signaling pathway (IGF1, PTEN and IGFBP1) in the endometrium may link polycystic ovarian syndrome and endometrial cancer. Mol Cell Endocrinol 424: 94-101.
22. Tal R, Seifer DB, Grazi RV, Malter HE (2013) Angiopoietin – 1 and angiopoietin – 2 are altered in polycystic ovarian syndrome (PCOS) during controlled ovarian stimulation. Vascular Cell 5: 18-24.
23. Banker M, Garcia-Velasco JA (2015) Revisiting ovarian hyperstimulation syndrome: Towards OHSS free clinic. J Jum Reprod Sci 8: 13-17.
24. Wang L, Qi H, Baker PN, Zhen Q, Zeng Q, et al. (2017) Altered Circulating Inflammatory Cytokines Are Associated with Anovulatory Polycystic Ovary Syndrome (PCOS) Women Resistant to Clomiphene Citrate Treatment. Med Sci Monit 23: 1083-1089.
25. De Leo V, La Marca A, Petraglia F (2003) Insulin-lowering

agents in the management of polycystic ovary syndrome. Endocrine Reviews 24: 633-667.
26. Ortega HH, Veiga-Lopez A, Sreedharan S, del Luján Velázquez MM, Salvetti NR, et al. (2015) Developmental Programming: Does Prenatal Steroid Excess Disrupt the Ovarian VEGF System in Sheep? Biol Reprod 93: 58.
27. Ben Salem A, Megdich F, Kacem O, Souayeh M, Hachani Ben Ali F, et al. (2016) Vascular endothelial growth factor (VEGFA) gene variation in polycystic ovary syndrome in a Tunisian women population. BMC Genomics 17: 748.
28. Didion SP (2017) Cellular and Oxidative Mechanisms Associated with Interleukin-6 Signaling in the Vasculature. Int J Mol Sci 18: E2563.
29. Zafari Zangeneh F, Naghizadeh MM, Masoumi M (2017) Polycystic ovary syndrome and circulating inflammatory markers. Int J Reprod Biomed (Yazd) 15: 375-382.
30. Szczuko M, Zapałowska-Chwyć M, Drozd A, Maciejewska D, Starczewski A, et al. (2018) Changes in the IGF-1 and TNF-α synthesis pathways before and after three-month reduction diet with low glicemic index in women with PCOS. Ginekol Pol 89: 295-303.
31. Brazert M, Pawelczyk LA (2015) Insulin-like growth factor-1 isoforms in human ovary. Preliminary report on the expression of the IGF-1 gene in PCOS patients and healthy controls. Ginekol Pol 86: 890-895.
32. Vainer N, Dehlendorff C, Johansen JS (2018) Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer. Oncotarget 9: 29820-29841.
33. Zhou F, Shi LB, Zhang SY (2017) Ovarian Fibrosis: A Phenomenon of Concern. Chin Med J (Engl) 130: 365-371.
34. Zhao M, Chang C, Liu Z, Chen LM, Chen Q (2010) The level of vascular endothelial cell growth factor, nitric oxide, and endothelin-1 was correlated with ovarian volume or antral follicle counts: a potential predictor of pregnancy outcome in IVF. Growth Factors 28: 299-305.
35. Visse R, Nagase H (2003) matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function and biochemistry. Circ Res 92: 827-839.
36. Piltonen TT, Chen JC, Khatun M, Kangasniemi M, Liakka A, et al. (2015) Endometrial stromal fibroblasts from women with polycystic ovary syndrome have impaired progesterone-mediated decidualization, aberrant cytokine profiles and promote enhanced immune cell migration in vitro. Hum Reprod 30: 1203-1215.
37. Rezvanfar MA, Saadi HAS, Gooshe M, Abdolghaffari AH, Baeeri M, et al. (2014) Ovarian aging-like phenotype in the hyperandrogenism-induced murine model of polycystic ovary. Oxidative med Cell Long, ID 948951.
38. Long X, Li R, Yang Y, Qiao J (2017) Overexpression of IL-18 in the proliferative phase endometrium of patients with polycystic ovary syndrome. Reprod Sci 24: 252-257.