Impact of metformin versus metformin plus omega_3 on inflammatory outcomes in a tester of Iraqi women with PCOS

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Inflammation, polycystic ovarian syndrome, IL-18, CRP.

ABSTRACT
Background: Polycystic ovary syndrome signifies a state of chronic inflammation, which is partly caused by excessive visceral adipose tissue and the pro-inflammatory mechanisms associated with it. Interestingly, even normal-weight women with PCOS exhibit long-lasting low-flat inflammation characterized via raised ranks cytokines of pro-inflammatory. This can be attributed to the presence of surplus visceral adipose tissue and intraperitoneal fat depots in normal-weight PCOS patients as well.
Aim of the study: To estimate the influence of metformin and omega-3 treatment on serum ranks of IL-18 and highly sensitive CRP in females with PCOS.
Patients and methods: The present interventional prospective study included 90 sick with an age assortment of 18 to less than 40 years. Those patients were analyzed with polycystic ovarian disease (PCOS) founded on Rotterdam principles (Rotterdam, 2004) by 2 specialists in obstetrics and gynecology. The patients were recruited from the Maternity and Pediatrics Teaching Hospital in Adiwaniyah Province, Iraq. The training is outmoded back to September the 21st 2023 and extended to March 31st 2024.
Results: Following treatment, both metformin+Omega3 and metformin alone were able to reduce mean IL-18 significantly ($p < 0.001$ versus $p < 0.001$, separately); however, the amount of decrease caused by combination was more than that caused by metformin alone significantly, 158.62 pg/ml versus 71.14 pg/ml , respectively ($p = 0.048$). Both metformin+Omega3 and metformin alone were able to reduce mean hs-CRP significantly ($p < 0.001$ versus $p < 0.001$, respectively); however, the amount of decrease caused through combination was more than that caused by metformin alone significantly, 157.69 mg/L versus 61.27 mg/L , separately ($p = 0.032$).
Conclusion: The use of Omega3 has an efficient and safe synergistic effect when added to metformin in reducing IL-18 and hs-CRP in sick with PCOS, thus, suggesting an anti-inflammatory property for such combination.

1. Introduction

PCOS is commonly attended via fatness and insulin resistance (IR), touching approximately 65-80% of completely sick (Al-Jefout et al., 2017). It is widely recognized that hyper-insulinemia, hyper-androgenism, and fatness in this condition mutually support every additional (Nehir Aytan et al., 2016). However, PCOS also signifies a formal of long-lasting inflammation (Patel, 2018), which is partly triggered via excessive visceral adipose tissue and the pro-inflammatory mechanisms associated with it. Interestingly, even normal-weight women with PCOS exhibit long-lasting low-flat inflammation characterized via raised ranks of pro-inflammatory cytokines. This can be accredited to the existence of remaining visceral adipose tissue and intra-peritoneal fat sidings in normal-weight PCOS sick as well (Regidor et al., 2020).

New research trainings have recognized two key features, specifically BMI and IR, that help as important analysts for raised ranks of CRP (C-reactive protein) and white blood cells (Rudnicka et al., 2020). Moreover, persons with polycystic ovary syndrome (PCOS) have been institute to take a specific pro-inflammatory genotype measured via changes in the genes accountable for TNF receptor, TNF-α, and IL-6 (Talaat et al., 2016). Additionally, hyperandrogenemia in PCOS not merely donates to augmented visceral adiposity however likewise productions a role in inflammatory manners. The further androgens motivate the stimulation of mononuclear cells (MNC), chief to

heightened creation of reactive oxygen species (ROS) and the stimulus of NFκB. Therefore, this activation progresses the appearance cytokines of pro-inflammatory, for instance TNF-α, IL-6, and Il-1. It is worth noticing that TNF-α and IL-6 stay documented as mediators of IR, and hyper-androgenemia has been foundation to negatively impact pathway signaling of the insulin-mediated IRS-PI3K-Akt (Zhang et al., 2018).

The metabolic appeals in PCOS and long-lasting inflammation are thoroughly linked through these associations. Moreover, there is a linking among irregular ovarian purpose and augmented infiltration of macrophages in the ovary, foremost to greater appearance of TNF-α, IL-6, and IL-8. This, in turn, activates signaling pathways of pro-inflammatory (Xie et al., 2016; Skaznik-Wikiel et al., 2016).

IL-18, also known as Interleukin-18, is a cytokine that was first identified via Okamura and colleagues in the late 20th century. Firstly, it stayed characterized as a factor that induces interferon-gamma (IFNγ) production. This powerful pro-inflammatory cytokine has been found to be elevated in various low-grade inflammatory environments, including fatness, metabolic disease, prediabetes, hypertension,type 2 diabetes, dormant auto-immune diabetes of the grownups, and dyslipidemia, as reported by Kabakchieva et al. in 2022. IL-18 elevation was detected in PCOS patients, as reported by Kabakchieva et al. in 2022. However, the inflammatory aspect of PCOS remains a subject of debate due to the wide range of findings reported. Consequently, there is inadequate evidence to definitively settle the matter. Some authors argue that the link among PCOS and little score inflammation is not entirely established and should be examined next categorizing contributors based on (BMI) or additional relevant parameters, using medically significant thresholds, as suggested by Duleba and Dokras in 2012.

2. Methodology

The present interventional prospective study included 90 sick with an age assortment of 18 to less than 40 years. Those patients were identified with (PCOS) founded on Rotterdam principles (Rotterdam, 2004) by 2 specialists in obstetrics and gynecology. The patients were recruited from the Maternity and Pediatrics Teaching Hospital in Adiwaniyah Province, Iraq. The training is outmoded back to September the 21st 2023 and extended to March 31st 2024. Pregnant women, women with chronic medical illness, women with hyperprolactinemia and women with thyroid disease were excluded from this study. Women were categorized into two groups: the first group (n = 45), received metformin as 500 mg per-oral (bid) for 90 days duration and omega 3 for three months; the second group (n = 45) received metformin as 500 mg per-oral (bid) for 90 days duration. Serum IL-18 and highly sensitive CRP (hs-CRP) were estimated before and after treatment using ELISA method (Elabscience, USA) and the procedure and calculations of results was based on the instructions outlined by the providing company.

The training stayed appropriate via the principled appreciation committee of College of Medicine/ University of Al-Qadisiyah. All participants were informed to give an oral consent next full design of the aims and the processes of the current training.

Data stayed analyzed and presented using SPSS (version 23, IBM, Chicago, USA) and Microsoft Office Excel 2010. Quantifiable variables stayed articulated as mean, standard deviation and assortment. Autonomous testers t-test was used to parallel means among two autonomous groups. Paired t-test stayed used to parallel means among two linked grouping earlier and next treatment.

3. Results and discussion

Comparison of mean age between Metformin +Omega3 grouping and Metformin Grouping is revealed in table 1. There stayed no important alteration in mean age among study groups, 26.11 ±3.98 years versus 26.71 ±5.77 years, respectively (p = 0.567). Comparison of mean (BMI) among metformin +omega3 group and metformin grouping before and after treatment is revealed in table 2. Before starting treatment, there stayed no important in mean BMI among training groups, 30.10 ±2.92 kg/m2 versus 30.42 ±2.75, respectively (p = 0.595). Following treatment, both metformin

+Omega3 and metformin alone were able to reduce mean BMI significantly ($p < 0.001$ versus $p < 0.001$, separately); however, the amounts of decrease in both groups stayed comparable with no significant difference, 2.30 kg/m2 versus 1.78 kg/m2, separately ($p = 0.179$). Appraisal of mean serum ranks of IL-18 among metformin +omega3 group and metformin Group before and after treatment is exposed in table 3. Before starting treatment, there stayed no important in mean IL-18 among training groups, 373.69 ±169.76 pg/ml versus 307.64 ±202.68 pg/ml, respectively ($p = 0.097$). Following treatment, both metformin+Omega3 and metformin alone were able to reduce mean IL-18 significantly ($p < 0.001$ versus $p < 0.001$, separately); however, the amount of decrease caused by combination was more than that caused by metformin alone significantly, 158.62 pg/ml versus 71.14 pg/ml , respectively ($p = 0.048$). Comparison of mean serum levels of hs-CRP between metformin +omega3 group and metformin Group before and after treatment is shown in table 4. Before starting treatment, there stayed no important in mean hs-CRP among study groups, 367.33 ±162.06 mg/Lversus 301.29 ±202.46 mg/L, separately ($p = 0.091$). Following treatment, both metformin+Omega3 and metformin alone were able to reduce mean hs-CRP significantly ($p < 0.001$ versus $p < 0.001$, respectively); however, the amount of decrease caused via combination was more than that caused by metformin alone significantly, 157.69 mg/L versus 61.27 mg/L , respectively ($p = 0.032$).

Table 3.1: Appraisal of mean age among Metformin +Omega3 grouping and Metformin Group

Characteristic	**Metformin +Omega3** ***n* = 45**	**Metformin grouping** ***n* = 45**	***p***
Age (years)			
Mean ±SD	26.11 ±3.98	26.71 ±5.77	0.567 I NS
Range	21 -36	18 -38	

Table 2: Appraisal of mean body mass index (BMI) among metformin +omega3 group and metformin grouping before and after treatment

Characteristic	**Metformin +Omega3** ***n* = 45**	**Metformin Group** ***n* = 45**	***p***
BMI before (kg/m^2)			
Mean ±SD	30.10 ±2.92	30.42 ±2.75	0.595 I NS
Range	26.08 -35.77	25.84 -35.38	
BMI after (kg/m^2)			
Mean ±SD	27.80 ±2.87	28.64 ±3.00	0.179 I NS
Range	24.15 -34.17	25 -34.13	
Difference in mean	2.30	1.78	
p	< 0.001 Pa ***	< 0.001 Pa ***	

Table 3: Appraisal of mean serum levels of IL-18 among metformin +omega3 grouping and metformin grouping before and after treatment

Characteristic	**Metformin +Omega3** ***n* = 45**	**Metformin Group** ***n* = 45**	***p***
IL-18 before (pg/ml)			
Mean ±SD	373.69 ±169.76	307.64 ±202.68	0.097 I NS
Range	130.92 -632.39	123.81 -746.24	

IL-18 after (pg/ml)			
Mean ±SD	215.07 ±88.52	236.50 ±180.88	0.048 I *
Range	96.15 -348.66	15.6 -631.68	
Difference in mean	158.62	71.14	
p	< 0.001 Pa ***	< 0.001 Pa ***	

Table 4: Appraisal of mean serum ranks of hs-CRP among metformin +omega3 grouping and metformin grouping before and after treatment

Characteristic	**Metformin +Omega3** ***n* = 45**	**Metformin Group** ***n* = 45**	***p***
hs-CRP before (mg/L)			
Mean ±SD	367.33 ±162.06	301.29 ±202.46	0.091 I NS
Range	129.46 -634.45	121.28 -749.01	
hs-CRP after (mg/L)			
Mean ±SD	209.64 ±85.63	240.02 ±181.78	0.032 I *
Range	96.2 -322.68	23.61 -635.11	
Difference in mean	157.69	61.27	
p	< 0.001 Pa ***	< 0.001 Pa ***	

Discussion

In this training it was detected that mutually metformin +Omega3 and metformin alone were able to reduce mean BMI significantly and the amounts of reduction in both groups were comparable indicating that no added effect can be obtained with administration of omega-3 in addition to metformin when weight reduction is intended in women with PCOS.

In line with current observation, the meta-analysis made by **(Zhou et al., 2023)** has shown that omega-3 alone failed to produce changes in mean BMI. In addition, **Vine et al.** in 2021 observed that adding omega-3 to metformin was comparable to use of metformin alone with respect to BMI reduction, an observation that is similar to that made in the current study. Therefore, is can be suggested that weight reduction in females with PCOS enrolled in this training stayed mainly attributed to metformin. Based on the training of **(Glueck et al., 2004)** a 5.8% decrease in mean BMI was attributed to metformin alone in women with PCOS. Presently available evidence indicates that the alteration in weight linked with metformin is probably attributable to reduced calorie consumption rather than heightened energy expenditure. Metformin seems to influence appetite control both straight and incidentally through its gastrointestinal side properties. **(Yerevanian and Soukas, 2019).**

In the present study, both metformin+Omega3 and metformin alone were able to reduce mean IL-18 and mean hs-CRP significantly and the amount of reduction caused by combination was more than that caused by metformin alone significantly. Raise of IL-18 and additional inflammatory bio-markers, for instance C-reactive protein (CRP) **(Elci et al., 2017; Dawood et al., 2018)** was observed in PCOS sick and the inflammatory environment of PCOS is highly accepted **(Kabakchieva et al., 2022)**. **Al-Qadhi et al.** in 2017 performed a study on 30 patients with PCOS aiming at exploration of role of metformin in changes of serum IL-18 and they reported significant reduction in serum IL-18; therefore, current study finding is in agreement with **Al-Qadhi et al. (2017)** study.

These findings align with those conducted via Khadiga El-Sayed Ali (2008), who investigated 40 PCOS sick in a retrospective non-placebo trial and observed a decrease in IL18 levels subsequent metformin management. Similarly, Sherif F. EL Mekkawi et al. (2010) reported a reduction in IL6 and IL18 levels in PCOS sick undergoing metformin therapy. Furthermore, Heutling et al. (2006) demonstrated a important rise in IL6 and IL18 in PCOS sick, with metformin management resulting

in improvements in metabolic and endocrine profiles, as well as menstruation. Trainings take proposed that metformin mitigates inflammatory responses by inhibiting nuclear factor κB (NFκB), a protein that triggers the stimulation of the gene responsible for cytokine production, via AMP-activated protein kinase (AMPK)-dependent and independent pathways. **(Saisho, 2015)**.

A recent experimental study has shown Supplemented with omega-3 was found to considerably reverse the increased appearance of IL-18 mRNAs in PCOS mouse ovaries (Zhang et al., 2023). Multiple reports suggest that n-3 PUFA can ameliorate inflammatory conditions in PCOS sick (Jamilian et al., 2018; Talari et al., 2018). In a training, concurrent supplemented of 3 g/day omega-3 from fish oil with 50000 IU vitamin D markedly reduced serum high-sensitivity C-reactive protein (hs-CRP) levels and downregulated IL-1 gene appearance, although it did not significantly affect gene appearance of TNF-α, IL-8, or transforming growth factor beta (TGF-β) (Jamilian et al., 2018). In additional investigation, supplementation with flaxseed oil omega-3 fatty acids led to a notable decline in hs-CRP (Mirmasoumi et al., 2018), while Rahmani et al. (2017) illustrated that consuming 1 g/day omega-3 fatty acids from fish oil for 12 weeks caused in the upregulation of PPAR-γ and the downregulation of gene appearance of IL-1 and interleukin-8 (IL-8) (Rahmani et al., 2017). Clearly, the common of prior studies underscore the effectiveness of n-3 PUFA supplemented in alleviating inflammatory conditions in PCOS sick.

It appears that the changes in the appearance of genes accountable for coding cytokines and inflammatory intermediaries contribute to the anti-inflammatory properties of n-3 PUFAs. This implies that pathways of n-3 PUFAs influence signaling, thereby regulating gene appearance in inflammatory cells. Additional apparatus through which EPA and DHA may impact the activation of (NF-κB) and the transcription of pro-inflammatory genes is associated with PPAR-γ. PPAR-γ functions as a transcription factor with anti-inflammatory properties, likely due to its inhibition of NF-κB translocation to the nucleus. **(Salek et al., 2019)**.

4. Conclusion and future scope

The use of Omega3 has an efficient and safe synergistic effect when added to metformin in reducing IL-18 and hs-CRP in sick with PCOS, thus, suggesting an anti-inflammatory property for such combination.

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