AN OVERVIEW ABOUT POSSIBLE CORRELATIONS BETWEEN VITAMIN D3 AND PROBIOTICS WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects woman's ovaries and reproductive system. PCOS is considered a challenging disorder for the physicians due to the continuous need for treatment modifications based on the patient's needs and preferences throughout her lifetime. The main cause of PCOS is unknown but a combination of several parameters including genetic and/or epigenetic factors, environmental factors, exposure to high levels of androgen prenatally and the hypothalamic pituitary-ovarian axis and adrenal dysfunction may play a role in the onset of the syndrome. PCOS is characterized by many metabolic, reproductive and hormonal disorders. Also, early diagnosis and treatment have a great importance to prevent many complications such as insulin resistance (IR), obesity, diabetes mellitus (DM), infertility, cardiovascular disease and even endometrial cancer. In the ovary, vitamin D was found to stimulate the production of progesterone and estradiol. Also, the presence of vitamin D3 in the follicular fluid and the expression of VDR in granular cells were confirmed. This fact suggests that vitamin D3 may be important in folliculogenesis, but it has not yet been clearly confirmed. Scientists claimed that, similarly to skin cells, in granular cells, vitamin D3 can stimulate the activity of aromatase, responsible for the conversion of androgens to estrogens, which would prove the role of vitamin D3 in folliculogenesis and ovulation. Moreover, vitamin D deficiency was reported to be common in women with PCOS. So, it was suggested that the metabolic changes in PCOS are related to dysfunction of vitamin D and calcium metabolism, which is important in follicular development and normal glucose metabolism. The relationship between PCOS and the gut microbiomes has emerged recently, and they were thought to play a role in the development of the syndrome. Environmental factors that drive the gut microbial community to become dysbiotic were supposed to make them playing a pathogenic role in the onset and progression of PCOS. Distinct microbiota were responsible for distinct pathogenic elements of PCOS. Women with PCOS were reported to have altered intestinal flora compared to healthy controls. This alteration was related to a decrease in α diversity and changes in β diversity. It has been found that there is a link between lower alpha diversity of gut microbiota and obesity, which is one of the most important co-morbidities of PCOS in women

Keywords: Vitamin D3, Probiotics, Polycystic Ovary Syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects woman's ovaries and reproductive system. PCOS is considered a challenging disorder for the physicians due to the continuous need for treatment modifications based on the patient's needs and preferences throughout her lifetime. The main cause of PCOS is unknown but a combination of several parameters including genetic and/or epigenetic factors, environmental factors, exposure to high levels of androgen prenatally and the hypothalamic pituitary-ovarian axis and adrenal dysfunction may play a role in the onset of the syndrome **(1).**

PCOS is characterized by many metabolic, reproductive and hormonal disorders. Also, early diagnosis and treatment have a great importance to prevent many complications such as insulin resistance (IR), obesity, diabetes mellitus (DM), infertility, cardiovascular disease and even endometrial cancer **(2)**.

Clinical features and major complications of PCOS:

The clinical presentation of PCOS varies widely and involves interrelated metabolic, reproductive, and psychological impairments. Women with PCOS often seek care for menstrual disturbances as (oligomenorrhea, amenorrhea and prolonged menstrual bleeding), clinical manifestations of hyperandrogenism (HA) (usually manifests as hirsutism which occurs in up to 70%, acne, and/or alopecia) and infertility which affects 40% of women with PCOS **(3)**.

Also, in a cross-sectional study conducted by **Joham et al. (4)**, infertility was recorded in 72% of women with PCOS compared to 16% in women without PCOS and that study found a significant higher use of hormonal fertility treatments among women with PCOS.

In addition, spontaneous abortion was reported to occur more frequently in PCOS with incidences ranging from 42%–73% which was explained by poor quality of oocytes and hyperinsulinemia-linked miscarriages **.**Moreover, even during pregnancy, PCOS patients were found to be at a higher risk of gestational diabetes and preeclampsia when compared to control females without PCOS **(5)**.

As regards the metabolic symptoms of PCOS, HA was suggested to be the beginning of a vicious cycle of metabolic disorders in PCOS patients. A positive correlation was observed between PCOS diagnosis in women and abdominal fat deposition. Hyperglycemia was supposed to be another metabolic disorder associated with PCOS. Dyslipidemia was considered one of the most common metabolic disorders identified in females with PCOS. Also, PCOS has been linked to significantly higher blood pressure compared to normal controls, independently of weight/obesity **(6).**

Diagnostic Criteria for PCOS

The diagnostic criteria for PCOS have been divided into numerous categories:

1-The National Institutes of Health (NIH) Criteria:

NIH proposed the diagnostic criteria for PCOS in 1990 which involved chronic anovulation, clinical and/or biochemical signs of HA and exclusion of other causes of HA such as 21-hydroxylase-deficient congenital adrenal hyperplasia, androgen secreting tumors, hyperprolactinemia, Cushing's disease, thyroid disorders and premature ovarian failure **(7)**.

2-Rotterdam Criteria:

The Rotterdam criteria were defined by the Rotterdam consensus conference held in 2003 and are currently the most used, but usage varies based on the nation and medical specialties. Two out of three criteria are needed to make the diagnosis after exclusion of the other causes for HA. The criteria include (1) oligo-amenorrhea and/or anovulation, (2) clinical and/or biochemical signs of HA, (3) polycystic ovarian morphology (PCOM) by ultrasonography (described by presence of at least one ovary has an ovarian volume greater than 10 mL and at least one ovary has an estimated ten small cysts, with diameters ranging from 2 to 9 mm). According to Rotterdam criteria, there are 4 phenotypes of PCOS: Phenotype A with oligo/anovulation, HA and PCOM, phenotype B with oligo/anovulation and HA, phenotype C with HA and PCOM, and phenotype D with oligo/anovulation and PCOM **(8)**.

Unlike the other criteria, the Rotterdam criteria do not require the presence of irregular menstrual cycles as a fateful symptom for PCOS diagnosis, but rather consider women with HA and PCOM as PCOS cases. The cause behind this diagnostic consensus is to widen the inclusion criteria and to recognize that PCOS does not represent a particular entity, but rather occurs in a range of heterogeneous disorders **(9)**.

However, Rotterdam criteria failed to take into consideration the metabolic status of the patients, which is sometimes reflected in increased body

mass index (BMI) and obesity in some women with PCOS **(10)**.

3-Androgen Excess and PCOS Society (AES-PCOS) Criteria:

The AES-PCOS recommended diagnosing PCOS in 2006 by the presence of 3 features: (1) clinical and/or biochemical signs of HA, (2) ovarian dysfunction (oligo/anovulation and/or PCOM), (3) exclusion of other causes of HA **(11)**.

The AES-PCOS criteria are similar to the NIH criteria in that they consider HA as a necessary component for PCOS diagnosis but unlike the Rotterdam criteria, they considered PCOM with ovulatory dysfunction (Phenotype D) alone does not qualify a patient for diagnosis according to the AES-PCOS criteria. So, the AES-PCOS criteria were considered more inclusive than the NIH version, but less than the Rotterdam criteria **(12)**.

Different PCOS phenotypes have been shown to carry various risks for metabolic problems. Women with both HA and ovulatory failure or with classic PCOS phenotypes as defined by the NIH, were reported to be typically characterized by increased prevalence of metabolic syndrome, central obesity, dyslipidemia, and pre-diabetes. So, the metabolic traits of PCOS phenotypes have been examined, however, it is still unclear which phenotype is most closely related to metabolic risk and the effect of racial differences on PCOS's reproductive and metabolic characteristics **(13)**.

Limitations of the currently used diagnostic criteria:

The study by **Peña et al. (14)** showed that the Rotterdam criteria did not take into consideration the difference between adult and adolescent female physiology. Diagnosis in adolescents might be challenging due to the overlap of diagnostic features of PCOS with normal puberty physiology so the Rotterdam criteria would result in an over-diagnosis of adolescents with PCOS and modifications should be made to the diagnostic criteria in adolescents. For example, for adolescents with suspected PCOS, two essential criteria should be only considered to diagnose PCOS which include irregular menstrual cycles and clinical and/or biochemical HA but PCOM should not be considered a criterion for PCOS diagnosis during the first 8 years of menarche. If only one of the two criteria is found, adolescents should be treated as cases at high risk of PCOS and should receive adequate medical follow up and symptomatic management.

Additionally, ultrasonographic evaluation of ovarian morphology and PCOM might not be possible in adolescents because transvaginal ultrasound might be inaccessible due to virginity combined with possible insufficient imaging by abdominal ultrasound due to abdominal obesity **(15)**.

Moreover, Rotterdam criteria established the diagnosis of PCOS based on PCOM and chronic anovulation without evidence of HA, while the NIH and AES-PCOS criteria perceive HA as the center of the PCOS diagnosis process. So, the heterogeneity between diagnostic criteria was considered a source of over and/or under diagnosis of PCOS **(16)**.

Vitamin D:

Vitamin D, a fat-soluble vitamin, was discovered in 1921. It acts in the body like a hormone with a multitude of functions. Vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) are particularly important for humans. Vitamin D2 is mainly found in plant-based foods, while vitamin D3 is abundant in meat products. The main source of vitamin D is solar ultra violet radiation, which converts 7-dehydrocholesterol to provitamin D in the skin **(17).**

Vitamin D receptors and main functions:

VDRs are present in numerous tissues and organs, including the intestines, bones, heart, brain, prostate, and mammary gland. VDR activation in organs such as the intestines, bones, kidneys, and parathyroid glands is mainly for the regulation of the concentration of calcium and phosphate in the blood **(18).**

Additionally, it was suggested that there is a relationship between low vitamin D levels and an increased probability of the development of such diseases such as, cancer, autoimmune diseases, Addison's disease, Graves' disease, immune disorders, atherosclerosis, ischemic heart disease, psychiatric diseases, and neurodegenerative disease. Vitamin D deficiency is considered a worldwide problem, regardless of age, gender and race. In some European countries, the deficiency of this vitamin was found in 90% of adults, children and adolescents. Also, when admitted to the intensive care unit, many critically ill patients (between 50% and 90%) were found to have low vitamin D levels**.** In general, prevalence of vitamin D deficiency in most population is reported to be 20-48%, but is relatively higher in women with PCOS (approximately 67-85%) **(19).**

Vitamin D and PCOS:

Vitamin D deficiency was reported to be common in women with PCOS. So, it was suggested that the

metabolic changes in PCOS are related to dysfunction of vitamin D and calcium metabolism, which is important in follicular development and normal glucose metabolism**.** Also, vitamin D deficiency has been reported to have strong association with oxidative stress and low-grade inflammation in PCOS patient **(20).**

Additionally, it was found that obese women with PCOS had lower vitamin D levels than those who were lean which may be due to lipophilic vitamin sequestration in adipose tissue, as well as lower exposure of obese people to sunlight or may be due to polymorphisms in the VDR gene that may determine susceptibility to PCOS. Furthermore, it was reported that vitamin D supplementation in PCOS patients resulted in a significant improvement in some PCOS hormonal parameters as serum total testosterone, high sensitivity C-reactive protein, total antioxidant capacity, and malondialdehyde (MDA), but no significant effect and enough studies were found on other parameters as free testosterone, DHEAS, sex hormone-binding globulin, free androgen index, nitric oxide, and total glutathione levels **(21).**

Also, it has been claimed that there is a relationship between vitamin D and the pro-inflammatory advanced glycation end products (AGEs) and their anti-inflammatory soluble receptors, sRAGE (AGE-RAGE axis). AGEs are highly reactive pro-inflammatory molecules that are formed naturally by non-enzymatic alteration of proteins, lipids, and nucleic acids by glucose or by ingestion of a variety of fast foods. They were reported to be elevated in chronic diseases such as DM, IR, aging, oxidative stress, and PCOS. After binding of AGEs to RAGE inside the cells like ovaries, sequence of events leading to cellular inflammation and apoptosis happens while extracellular or soluable RAGEs which bind to AGEs before their attachment to cellular RAGEs considered as anti-inflammatory receptors **(22).**

Also, it was reported that women with higher follicular fluid vitamin D level were significantly more likely to achieve clinical pregnancy, but no study until now comparing follicular fluid vitamin D levels and pregnancy outcome in PCOS patients **(23).**

Probiotics:

The Nobel Prize winner for Medicine in 1908, Elie Metchnikof stated that "the dependence of friendly bacteria on food allows measures to be taken to modify the microbial composition of our body and thus replace the harmful ones". He claimed that the cause of aging is toxin released by the disintegration of certain bacteria in the intestine or by the degradation of components through the release of proteolytic enzymes from Clostridium species so that probiotics began to be used to improve the health of patients. In 1989, Fuller defined probiotics as a dietary supplement with live microbata which has a positive effect on the host by improving the microbial balance of the host's gut. Then, in 1991, he defined probiotics as single or mixed cultures of live microorganisms that have a beneficial effect on humans or animals and contribute to the improvement of the properties of their acquired endogenous microbial presence**.** In 1998, a group of scientists claimed that probiotics are food components composed of living microorganisms that have a beneficial effect on the health of humans **(24).**

One of the most accepted explanations to define probiotics was added by WHO which defined probiotics as microorganisms which, when administered in sufficient quantities, can improve health of the patient and avoid the dysbiosis and lead to the gut microbiota's eubiosis.The most used probiotics species are from Lactobacillaceae family (such as, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus johnsonii, Lactobacillus crispatus, Enterococcus faecium, plantarum subsp, Lacticaseibacillus rhamnosus), Bifidobacteria family (such as, Bifidobacterium animalis, Bifidobacterim lactis, Bifidobacterium infantis, Bifidobacterium adolescentis, Bifidobacterium breve, Bifidobacterium longum) and others (such as, E. coli, Saccharomyces, Aspergillus, Clostridium butyricum) **(25).**

Difference between Probiotics, Prebiotics and Synbiotics:

Probiotics are live microorganisms as mentioned before. Prebiotics are a non-digestible food component (typically high-fiber foods) that positively affects the host by specifically promoting the growth or activity of a select group of bacteria in the colon. Moreover, prebiotics are alimentary ingredients that found naturally in vegetable foods or can be produced by synthetic production through enzymatic conversion of sugars. The most used prebiotics are galattooligosaccarides (GOS) and inulin derivatives, such as fructooligosaccarides (FOS). The GOS derived from lactose are found in human milk and vaccines, but are also present as additives in many other foods, such as cereals and dairy products. GOS favors the proliferation strains from the Lactobacillaceae and Bifidobacteriaceae families, which are highly beneficial for the host's

health**.** Not all fibers are prebiotics and not all prebiotics are fibers **(26).**
Whereas, synbiotics are the mix of probiotics and prebiotics to obtain adequate concentrations to achieve a beneficial effect in the host's health **(27).**

Sources of probiotics:
Probiotics can be taken through dairy functional foods (such as yoghurt, cheese, ice cream, fermented food, olives, apple vinegar, kimichi or fermented cabbage, green beans, butter milk and others) and through non-dairy products or external supplementation. During preparation of probiotics drugs, the micro-encapsulation technique is used to maintain the best performance and to protect them from dangerous environmental conditions, such as acids, alkalinity, heat, humidity, and even the interaction with other compounds. Microencapsulation is a process through which droplets or microscopic particles of liquid or solid materials are surrounded, covered, or embedded in a continuous film of polymeric material, homogeneous or heterogeneous, to produce small capsules with useful properties **(28).**
Administration of probiotics was reported to be highly safe, but some tests have shown adverse effects associated with the administration of probiotics, as is common with any preparation or medication such as nausea, indigestion and abdominal discomfort (such as flatulence, and constipation), less commonly, infections can develop but the most serious side effects that have been reported are endocarditis and septicemia **(29).**

Importance of probiotics:
The gut microbiome has been referred to as a new human organ and is important for maintaining health. Microbial communities are found on a variety of environmental surfaces (such as gastrointestinal tract, skin, genitourinary system, upper and lower airway tract, etc). The colon is the most suitable habitat for various microbial populations due to its stable alkaline pH environment. The human microbiota, environment, genotype, gender and the individual's lifestyle (sedentary life, exercise, smoking, abuse substances, chemical compounds such as bisphenol A, etc.), are factors that were reported to interact with each other and influence health and the development of diseases and inflammation. The gut microbiota dysbiosis was suggested to activate a chronic irregular immune response and metabolic dysfunction resulting in the development of metabolic diseases, such as obesity, and the inflammatory response in various body tissues involving various systems, such as the CNS **(30).**
The microbiota-gut-brain axis (GBA) is considered a bidirectional regulatory pathway between the brain and the gastrointestinal tract, which might play an important role in maintaining homeostasis and immunity. Gut microbiota were suggested to influence the behavior, cognition, stress response and others via this axis. Probiotics were found to have a very important role in health and disease resistance. Probiotics must be taken in sufficient quantities through functional foods to maintain the microbiota eubiosis, protect against gastrointestinal pathogens, strengthen the immune system, control the normal levels of serum cholesterol, regulate the blood pressure, protect against the development of certain cancerous conditions, improve nutrient processing and the nutritional value of foods, promote synthesis of vitamins, enhance protein digestion, stimulate production of antimicrobial agents and aid in fighting infections **(31).**

Moreover, It was summarized the most important suggested benefits of probiotics on eubiosis, immune homeostasis, intestinal activity effects and metabolic effects as follow: (1) compete against endogenous pathogens as antibiotic's diarrhea, barrier against exogenous pathogens as travellar's diarrhea, (2) stimulate and regulate innate immunity, and play a role against autoimmune diseases and allergic reactions, (3) support the digestive process, help in integrity of intestinal epithelium, regulate mucous production and synthesis of vitamins as K, B2, B12, B6, (4) might play a role against cancers, help in cholesterol reduction, avoid skin diseases and cardiovascular problems. **(32).**

Additionally, many clinical trials showed that probiotics were useful in treating Crohn's and ulcerative colitis. The intake of probiotics increased short chain fatty acids that maintain the integrity of the intestinal mucosa, increased the inhibition of inflammatory response, and caused a significant increase in "friendly" bacteria living in the gut that will dominate the intestinal microbiota, resulting in a simultaneous reduction in the undesirable ones. Furthermore, one of the most important uses of probiotics is in treatment of diarrhea. The usage of probiotics was suggested to prevent diarrhea that occurs not only in undernourished children in developing countries, but also in acute diarrhea by decreasing the duration of diarrheal episodes. Probiotics species such as L. rhamnosus and S. Boulardii were

supposed to be effective both in diarrhea caused by antibiotic use and that caused by infections **(33).**
Regarding to the usage of probiotics in cancers, it has been found that probiotics have an anti-proliferative effect on cancer cells. The bio-mechanics that underlie the anti-cancer action were reported to be diverse and might include the suppression of microorganisms that produce and secrete mutagens and carcinogens, altering the metabolism of carcinogens, shielding DNA from oxidative damage, and immune system control. They have also been demonstrated to play a role in altering the expression of genes related to cell cycle regulation, infiltration and metastasis, the maintenance of cancer stem cells, and apoptosis and cell death. More research has revealed that probiotics might influence the signaling pathways that cause cancer **(34).**

Probiotics and PCOS:
The relationship between PCOS and the gut microbiomes has emerged recently, and they were thought to play a role in the development of the syndrome. Environmental factors that drive the gut microbial community to become dysbiotic were supposed to make them playing a pathogenic role in the onset and progression of PCOS. Distinct microbiota were responsible for distinct pathogenic elements of PCOS. Women with PCOS were reported to have altered intestinal flora compared to healthy controls. This alteration was related to a decrease in α diversity and changes in β diversity. In addition, the relative abundance of many bacteria, such as the Bacteroidaceae, S24-7, Ruminococcaceae, and Clostridiaceae families, was altered. Also, a decrease in beneficial bacteria (Lactobacilli and Bifidobacteria) and an increase in pathogenic bacteria (Escherichia and Shigella) were found**.** It has been found that there is a link between lower alpha diversity of gut microbiota and obesity, which is one of the most important co-morbidities of PCOS in women **(35).**

Recently, there are two different theories were proposed to find the link between gut microbata changes and HA in PCOS women. One theory supposed that gut dysbiosis caused by a high-fat diet and a high carbohydrate diet affects the gut barrier function, leading to IR, HA, and dysfunction of the ovaries. This theory strongly found a relationship between the diet and gut dysbiosis which is considered as driving forces from where pathogenic features of PCOS related to HA occur, however, it does not consider that despite differences in diet, occurrence of PCOS is also observed. The other theory was based on the fact that testosterone could affect the gut microbiota through a direct effect as a substrate for gut microbial enzymes and an indirect effect via activation of host androgen receptors or immune system regulation **(36).**
However, HA was proposed to cause gut dysbiosis according to the way used to induce PCOS in animals like rats especially PCOS model induced by letrozole. Treatment with letrozole resulted in a change in the abundance of some gut microbata species, but surprisingly after stopping the letrozole, restoring gut bacterial diversity was found. Moreover, it was reported that poor diet leads to gut microbiota dysbiosis and that may lead to an increase in the permeability of the gut mucosa, which in turn increases the passage of lipopolysaccarides from Gram-negative colonic bacteria into the blood stream. The resulting immune system activity disrupts insulin receptor function, elevating serum insulin levels, increasing the production of androgens in the ovaries, and interfering with normal follicle formation **(37).**

Also, dysbiosis in PCOS was reported to be related to mood disorders and GBA dysfunction. Glucagon like peptide 1 (GLP-1), which affects the gastro-intestinal system and the CNS via the vagus nerve, is disordered in PCOS patients. GLP-1 was reported to play a critical role in multiple functions such as delaying gastric emptying time, decreasing appetite, increasing satiety, promoting pancreatic islet cell proliferation, and stimulating insulin. PCOS patients were reported to suffer from depression, social phobias, anxiety, and aggressiveness. These symptoms were linked to a disrupted GBA **(38).**
The ovary, liver, skeletal muscle, and adipose tissue are some of the host tissues whose functions are altered in PCOS. The gut microbiota metabolize the substrates entering the gut via meals and produce compounds that may act directly on the intestines or enter systemic circulation and influence various host tissues. A few metabolites produced by the gut flora that are changed in PCOS include trimethylamine, short-chain fatty acids, inosine-5-monophosphate and secondary bile acids **(39).**

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