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Bidirectional Communication between Gut Microbiome and Polycystic Ovary Syndrome: Implications on Associated Metabolic Comorbidities

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Abstract

Objective: Polycystic Ovary Syndrome (PCOS) is a neuroendocrine and metabolic disorder with multifaceted etiology, prevailing in women who are of reproductive age, rendering dwindling conception rates and escalating infertility rates worldwide. The etiology of PCOS is unresolved, potentially caused due to a mixture of genetic and environmental factors supported by components of diet and lifestyle manifested in women as an endocrine and metabolic disorder. Recent advancements have however thrown light on the influence of the gut-brain axis and the Gut Micro-Biome (GMB) on various body functions. Endocrine, immune and metabolic dysfunctions, portrayed by abnormal steroidogenesis and gut-induced inflammation, influenced by dysbiosis of the gut, provides a plausible role to the gut microbiome in the pathophysiology of PCOS. Endocrine Disrupting Chemicals (EDCs) mimic endogenous hormones and interfere with homeostasis. EDCs can have a significant impact on the health of women, in particular with PCOS, owing to its increasing link with estrogen, testosterone, and weight gain and glucose metabolism. **Methods:** A thorough search was conducted onelectronic databases. Relevant literature, obtained through the search, were studied and summarized to address the effects of EDCs on the gut-microbiome and PCOS and the associated metabolic comorbidities. **Conclusion:** GMB is associated with various metabolic disorders inching towards comprehensive development of metabolic syndromes, thereby increasing risks of developing chronic obesity, infertility, Type 2 diabetes mellitus, cardiovascular disorders, and gynaecological cancers. Influence of EDCs on the gut-brain axis and there by the pathophysiology of PCOS, and the bifacial alliance between GMB and PCOS involving endocrine, immune and metabolic mechanisms open up a novel avenue in managing the effect of EDCs in PCOS women worldwide.

Keywords: Endocrine Disrupting Chemicals, Gut Microbiome, Metabolic Comorbidities, Polycystic Ovary Syndrome

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder affecting every 1 in 10 women of reproductive or child-bearing age around the world. PCOS is characterized by features assigned by the Rotterdam Consensus (2003) and the Androgen Excess and PCOS Society (2006). The Rotterdam criteria include oligo/anovulation, hyperandrogenism with the absence of any endocrinopathies[1] as an initial diagnosis of PCOS and

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the presence of polycystic ovaries as a third diagnostic prerequisite, thus establishing the requirement of two out of the three features, to be classified with PCOS[2]. The exact aetiology of PCOS is still unresolved but is often attributed to a variety of genetic, lifestyle, and environmental factors affecting the endocrine system. This chronic disease is an endocrine and metabolic disorder distinguished by irregular menstrual cycles, irregular ovulation (difficulty becoming pregnant), acne and hirsutism accompanied by a high risk of metabolic disorders such as insulin resistance (IR), type 2 diabetes (T2D), Cardio-Vascular Diseases (CVD), infertility, obesity, cancer and non-alcoholic fatty liver disease[3]. To develop treatments for this syndrome, it is vital to consider the endocrine and metabolic aspects of the disease focusing on the mechanisms of the metabolites involved in the maintenance of the reproductive system. Recent studies have indicated the involvement of the human GMB in the pathogenesis of PCOS and the possibility of internal microbe niches influencing PCOS phenotypes. The human gastrointestinal (GI) microbiome consists of a plethora of bacterial, archaea, phages, yeast, protozoa, and fungal species that exist in a symbiotic relationship within the gut of the body. The microbial community in the gut is termed as the "gut microbiota" and the multitude of genes present in the gut microbiota is termed as the "gut microbiome". Owing to advancements in genomic studies and metagenomic analysis, the composition of the microbes has been studied and attributed to the development of certain diseases such as neuro-psychological disorders, cancer, cardiometabolic disorders, and inflammatory bowel disease[4,5]. Development of the GMB can occur throughout the life of an individual; however, the establishment of the primitive GMB occurs during fetal development via digestion of amniotic fluid. The nature of the microbial colony is influenced by the characteristics of the microbe and gut habitat. Aero-tolerant and facultative bacterial colonizations are succeeded by obligate anaerobes and Bifidobacterium[6]. The major taxonomic groups residing within the gut include Firmicutes, Bacteroides, Proteobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria, and Actinobacteria[7]. Host environmental factors, pH, transit time, bile acids, digestive enzymes, and mucus, non-host factors (nutrients, medications), and bacterial factors (adhesion and metabolic capacity, enzymes) are displayed along the GI tract. The GMB confers properties to the immune system via signals generated for the development of T regulatory, and T helper (Th1 and Th2) cells[8] and Th17 cells which are involved in the regulation of the immune system and secretion of cytokines as a defense against foreign antigens. Metabolic functions of the GMB consist of bile acid transformations by microbial enzymes for cholesterol and glucose metabolism, amino acid synthesis, and vitamin production[9]. The metabolic activities of the microbiotaare imperative for the host metabolism by the generation of gases and Short-Chain Fatty Acids (SCFAs)[10]. The biochemical pathways are provided by the microbes and through fermentation, the major SCFAs produced include acetate, butyrate, and propionate which are essential for energy production and cholesterol synthesis. Diet is a well-known factor playing a role in the regulation of sex steroid metabolism. Several studies have demonstrated that a high-lipid and low-fibre diet is related to an increase in circulating androgen levels[11]. Various studies incorporating hyperandrogenism in women with PCOS indicates the potential roles of the GMB. The GMB was observed to be altered in humans with metabolic disorders such as obesity and T2D, and changes in the GMB were accompanied in women with PCOS and in rodent models. The total genus of the GMB decreased along with an increase in Firmicutes (related to obesity) in letrozole-induced PCOS in another study, highlighting the influence of the microbiome[12]. Alterations in the microbial environment correlate with hyperandrogenism in women with PCOS and letrozole-induced PCOS mouse models and indicate that a microbial imbalance or dysbiosis in the gut linked to hyperandrogenism might contribute to the development of PCOS. This article aims to primarily address the possibility of a bidirectional relationship between PCOS and the GMB, with an emphasis on understanding the gut-brain axis and the role of Endocrine-Disrupting Chemicals (EDCs) in contributing to dysregulation in PCOS and associated metabolic comorbidities (Figure 1).

2. Endocrine Disrupting Chemicals and Polycystic Ovary Syndrome

Endocrine Disrupting Chemicals (EDCs) are exogenous agents that alter endocrine function and have negative health consequences. The majority of EDCs are synthetic chemicals, but some are phytoestrogens found in natural foods. People are exposed to complex chemical

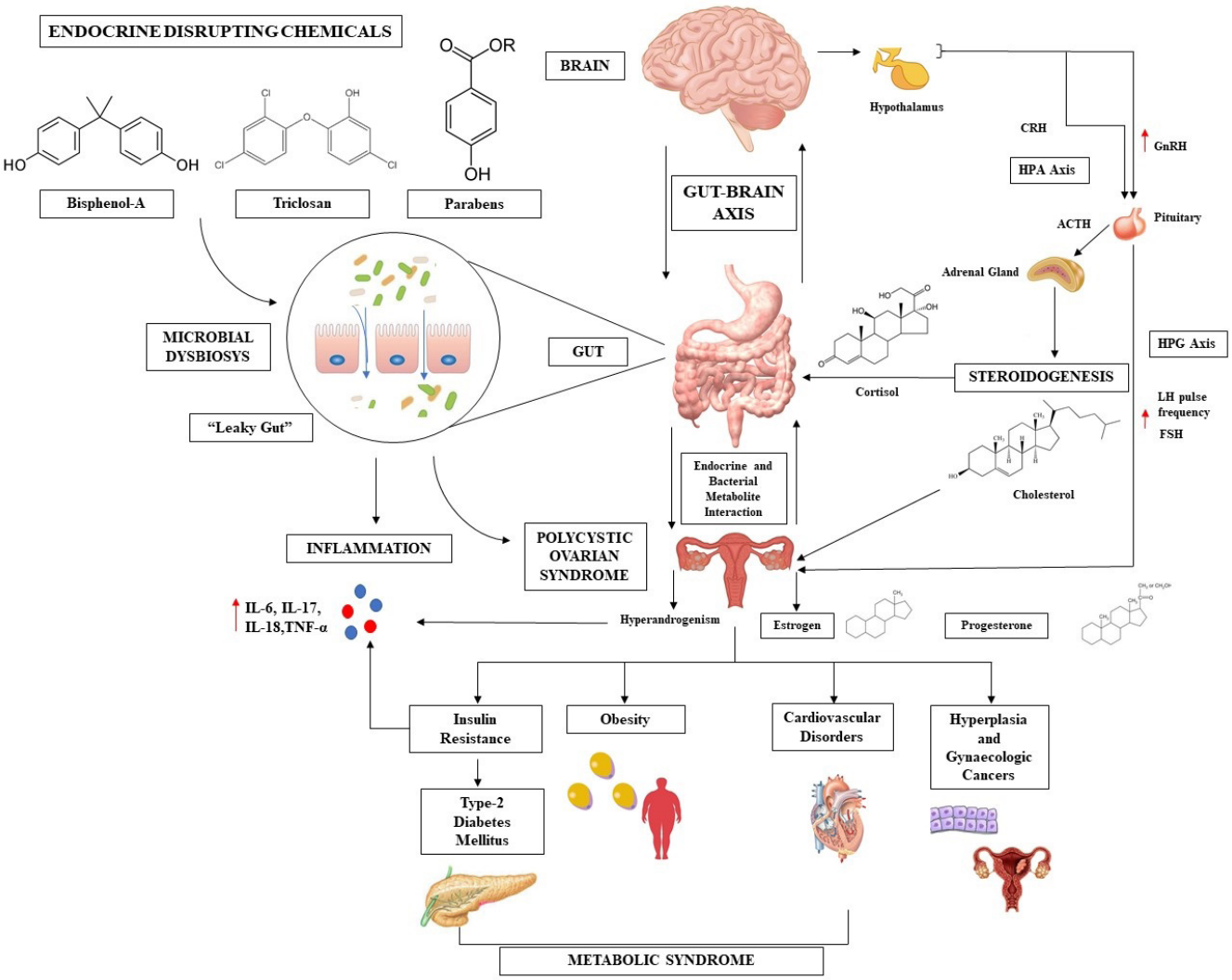

Figure 1. Schematic representation of influence of Endocrine Disrupting Chemicals (EDCs) on the bidirectional relationship between gut-brain axes in PCOS.

mixtures throughout life, and both hormone-dependent metabolic systems and brain function are affected by EDCs. Bis-Phenol A (BPA) is a popular endocrine disrupting environmental chemical that is widely used in manufacturing epoxy resins and polycarbonate plastics. BPA and BPA analogs have been banned due to concerns about the safety of this EDC in living organisms[13]. Tri-Clo-San (TCS) and Tri-Clo-Carban (TCC) (TCs) are chlorinated, broad-spectrum antimicrobial endocrine disrupting chemicals that are found in a wide variety of consumer and industrial products, as well as contaminated foods, and these EDCs are related to metabolic disorders such as obesity and diabetes[14]. Phthalates are environmental degradation products (EDCs) that are used as plasticizers in food processing and packaging, adhesives, personal care products, and cosmetics. Phthalates have been labeled as obesogens, meaning they contribute to obesity and overweight. Exposure to phthalates has been shown to alter glucose and lipid metabolism, increasing the risk of developing insulin resistance[15].

In various studies TCS and parabens (DEP) have been shown to alter microbiota. Hu *et al.*[24] looked at the effect of simultaneous exposure of low-dose DEP, methylparaben, and TCS on gut bacterial composition in adolescent rats and found that the exposed rats had a higher relative abundance of Bacteroidetes (Prevotella) and a lower relative abundance of Firmicutes (Bacilli)[16].

At sub-lethal concentrations, methylparaben changed the critical physiological and developmental landmarks such asheart rate and hatching rate in zebrafish. It has also been observed to cause anxiety-like behavior in the treated larvae as well as a reduction in exploratory behavior[17]. Shekhar *et al.*[18] showed that the *in utero* fetal exposure during development is positively correlated with maternal exposure to several chemicals and that EDCs are still present in the general population, including pregnant women and fetuses. BPA was studied to alter neurobehavioral effects in male and female Wistar rats and in both male and female offspring. The prenatal BPA exposure increased anxiety-like behavior in males and decreased exploratory behavior[19]. Hormone-dependent genital cancers, such as ovarian and endometrial, may be caused due to chronic exposure to low doses of EDCs. Long-term exposure to low concentrations of EDCs may potentiate the effects of other EDCs, as well as endogenous estrogens, and inhibit estrogen metabolizing enzymes[20]. EDCs with estrogenic properties may also cause sustained increase in LH (a "positive" feed-back loop), resulting in increased androgen production in the theca cells, which is a common feature in women with PCOS. BPA has been linked to ovarian and metabolic dysregulation and, hence, an investigation into its relation to PCOS, a condition that is characterized by both reproductive and metabolic abnormalities in imperative. In humans, elevated BPA concentrations are found in adolescents and adult PCOS women compared to reproductively healthy women, and they are positively correlated with hyperandrogenemia, suggesting that the chemical may play a role in the pathophysiology of PCOS. In genetically predisposed individuals, developmental exposure to specific EDCs may permanently alter neuroendocrine, reproductive, and metabolic regulation, favoring PCOS development, or it may accelerate and/or exacerbate the natural course of the syndrome on exposure throughout life[21]. The consequence of EDC exposure in PCOS women and its influence on associated metabolic comorbidities can thus be further analyzed by focusing on harnessing the GMB.

3. Gut-Brain Axis

A connection between the Central Nervous System (CNS) and the Enteric Nervous System (ENS) is forged by the bidirectional relationship generating an essential Gut-Brain Axis (GBA). The GBA is crucial for mediating peripheral intestinal functions directly or indirectly with cognitive and emotional centers of the brain. The peripheral intestinal functions controlled include immune activation, intestinal permeability, enteric reflex, and entero-endocrine signaling[22,23]. A multiplex communicative basis is established between the endocrine, immune and Autonomic Nervous (AN) systems. This relationship is dependent on the mediators of the Hypothalamic-Pituitary-Adrenal (HPA) axis and immune molecules like cytokines and chemokines. The efferent signals from the CNS to the gut, and the afferent signals from the intestinal lumen via the vagus, enteric and spinal nerves to the CNS are mediated by the ANS majorly influenced by the HPA axis that harmonizes the bidirectional responses. The neuroendocrine premise of PCOS is based on the influence of the Hypothalamic-Pituitary-Gonadal (HPG) axis where hyperactive Gonadotropin-Releasing Hormone (GnRH) neural circuit leads to abnormally high Luteinizing Hormone (LH) pulse frequency[24] affecting at least 10% of women of reproductive age. PCOS is typically characterized by the presence of at least two of the three cardinal features of hyperandrogenemia (high circulating androgen levels. Theneural and endocrine systems similarly impact the gut via influencing the function of intestinal effector cells such as epithelial cells, enteric neurons, smooth muscle cells, immune cells, interstitial cells of Cajal and enterochromaffin cells by the release of cortisol from the adrenal glands. Corticotropin-Releasing Factor (CRF) from the hypothalamus stimulates Adreno-Cortico-Tropic Hormone (ACTH) from the pituitary which activates the gut-brain relationship supplemented by environmental stress and elevated systemic pro-inflammatory cytokines[25]. Further evidence of this relationship is validated by the altered expression and turnover of neurotransmitters associated with the absence of microbial colonization; this colonization is vital for development and maturation of the CNS and ENS[26]. Inflammation aiding in gut-brain axis activation is considered as a causative driving force and resultant condition of PCOS. Elevated levels of pro-inflammatory cytokines such as interleukin 6 (IL-6), IL-17, and Tumor Necrosis Factor-α (TNF-α) in PCOS are indicative of a chronic low-grade inflammatory state. This is supported by cytokine release (TNF-α) from Mono-Nuclear Cells (MNCs) by glucose stimulation and hyperandrogenism, contributing to a state of IR in PCOS women[27]. Modulation of intestinal barrier is one of the major factors that can

alter the compartments of the gut. Various plausible mechanisms exist between interactions of the microbiota with GBA. Alterations in mucus and biofilm production, motility, intestinal permeability and immune function are viable interactions from the brain to gut microbiota. The gut microbiota to brain reciprocity is attained by mucosal immune regulation, bacterial metabolite secretion, modulation of enteric sensory afferents, production, expression and turnover of neurotransmitters and neurotrophic factors (serotonin, gamma-aminobutyric acid and brain-derived neurotrophic factor, respectively) and protection of intestinal barrier and tight junction integrity[28]. Gut barrier integrity alterations contribute to reproductive and metabolic defects in PCOS women due to a "leaky gut" caused by an alteration in tight junction integrity linked to states of inflammation is PCOS conceivably aided by Lipo-Poly-Saccharide (LPS) released by Gram-negative bacteria. ENS activity is influenced by catecholamine generation and local neurotransmitter release[29]. It is thus evident that diet and stress can induce variations in the gut microbiota, and the regulation of the HPA-GBA axis is essential for maintenance and homeostasis of the intestinal niche. Evidence of microbiome-GBA interactions is derived from the presence of dysbiosis with CNS disorders and GI disorders. Diet plays a significant role by influencing the intestinal microbiota of the human microbiome by impacting energy and immune homeostasis. Maintenance of the GMB can be ensured by low fat and sugar, and high fermentable or prebiotic diets to reduce systemic disease occurrences and improve the universal environment of the intestine[30]. Dysbiosis is an imbalance or disturbance within the microbial population that alters the functioning capacity of the GI tract. Dysbiosis is associated with many diseases such as irritable bowel syndrome, autoimmune disorders such as ulcerative colitis and gastric and colon cancers, thus vindicating the possibility of dysbiosis causing metabolic disorders affiliated to PCOS. An important study concerning the role of Glucagon-Like Peptide-1 (GLP-1) agonists on the gut-brain axis, was observed to be an effective therapeutic approach for obese women with PCOS[31]. The GI system modulates metabolism via orexigenic hormones (e.g., Ghrelin) and circulating anorexigenic peptides such as GLP-1, Gastric Inhibitory Peptide (GIP), Peptide YY (PYY) and Chole-Cysto-Kinin (CCK). On the other hand usage of metformin is associated with increase in ghrelin, PYY, GLP-1 and GIP levels in women with PCOS.

Microbiota can metabolize EDC to biologically active or inactive forms in a bidirectional interaction, and EDC can stimulate the proliferation and growth of certain bacteria. In *in vitro* and *in vivo* models, several EDCs have been shown to promote dysbiosis or inhibit bacterial growth[32]. Changes in the gut microbiota caused by EDCs may cause problems in various host systems. However, it is unclear whether EDC-induced metabolic disruptions in the host occur before or after change in the microbiome, or if metabolic disruptions are caused by EDC-induced microbiome changes[33]. In California mice perinatal genistein (phytoestrogen; an EDC) exposure may produce a negative impact on the offspring's microbiome-gut-brain axis. EDC can also alter the composition and metabolic activity of gut microbiota, resulting in EDC metabolite activity being disrupted or the toxicity of other contaminants metabolized by the gut microbiota being increased[34]. Furthermore, EDC exposure may alter the functions of the gut microbiota. Microbiota and their products may act as mediators of the contaminants' effects, eventually leading to the emergence of disorders and diseases. The possibility that EDC exposure causes changes in gut microbial composition has not yet been thoroughly investigated. However, these findings have established the need to understand the role of gut-brain axis in targeting the GMB as a potential therapeutic approach for generating neoteric treatments for PCOS.

4. PCOS-Associated Metabolic Comorbidities

PCOS consists of a myriad of clinical features such as hyperandrogenism, hirsutism, infertility or sub-fertility and menstrual irregularities. A wide range of PCOS women is, however, diagnosed with metabolic dysfunctions that are often manifested collectively as metabolic syndrome. Metabolic syndrome consists of an assemblage of metabolic disorders encompassing those manifested in PCOS such as abdominal obesity, IR, impaired glucose metabolism, hypertension and dyslipidemia. These disorders increase the risk of development of T2D, Coronary Heart Disease (CHD), Cardio-Vascular Diseases (CVD) and endometrial cancer[35]. Insulin-resistance provides an overlap between PCOS and metabolic syndrome, thereby associating obesity and its subsequent association with infertility as a risk factor of developing metabolic syndrome.

Abnormalities in gonadotropin secretion, weight problems, IR and hyperinsulinemia are factors that govern ovarian hyperandrogenism in PCOS[36], creating steroidogenesis a logical pathway for the treatment of PCOS and associated metabolic disorders. As previously understood, an interrelationship is observed between ovarian and adrenal steroidogenesis with the intestinal microbiota. The GMB assert their control over the host metabolism via an altered and defective gut barrier, bile acid metabolism, antibiotic use and a wide range of functioning metabolites[37]. The microbiota are established to be associated with metabolic syndromes' comorbidities such as obesity and T2D due to varying microbial compositions. Diversity and composition of the microbial niche influence host metabolism and hormonal levels, underpinning our focus on GMB's role in PCOS and its associated metabolic disorders. The emphasis of EDCs on the GMB as a mediator of metabolic comorbidities has drawn elaborate investigations[38]. Increase in metabolic diseases correlate with a simultaneous influx in generation and extensive utilization of EDCs. Altered gut bacteria have been strongly linked to changes in the microbiota metabolites. These changes increase the risk of tissue dysfunctions that might lead to obesity, insulin resistance, and cardiovascular disease[39]. Emerging evidences from a variety of studies correlate with the impact of EDCs on metabolism via several vital pathways, shedding light on the focus of EDCs on metabolic comorbidities.

4.1 PCOS-GMB: Obesity as a Comorbidity

Obesity is a common feature among PCOS women, indicating a predominant metabolic dysfunction in women of reproductive ages. It is, however, still undetermined as to whether obesity can lead to PCOS or obesity is a manifestation of pre-existent PCOS condition. Obesity is a probable cause of characteristics of IR and hyperinsulinemia and, hence, favour hyperandrogenism, and it has been observed that weight gain often precedes the onset of oligomenorrhea and hyperandrogenism[40]. It has been conceived that increased adrenal androgen level can be attributed to dysregulation of the HPA axis. The impact of steroidogenesis in obesity as a metabolic disorder can potentially be connected by cortisol activity impairment. A surge in the catabolism of cortisol might lead to a resultant hyperactivation of the HPA axis and consequently increased androgen formation. Consistent with obesity as a metabolic abnormality is the presence of IR, another metabolic state where sufficient concentrations of insulin produce insufficient biological responses among PCOS women[41]. Insulin stimulates ovarian steroidogenesis both in granulosa and thecal cells via interaction with its receptor and insulin-Like Growth Factor (IGF) receptor type 1; however, the role of insulin on aromatase enzyme has shown a variety of contradicting results[42]. Sex-Hormone Binding Globulin (SHBG) transports inactive forms of hormones in the blood, displaying a high binding affinity for testosterone and dihydrotestosterone and lower affinity for estrogen. Insulin decreases the levels of SHBG, liberating free androgens, thus reducing SHBG levels in insulin resistant PCOS women[43]. Fluctuating high lipid intake decreases serum SHBG levels^{-16}, rendering SHBG a factor to dietary factors, insulin, cortisol, estrogen, growth hormones, prolactin and IGF-1. Themetabolism of estrogen and cortisol is altereddue to decrease of formation of inactive estrogen metabolites and overstimulation of HPA axis[44]. Obesity is associated with an increased incidence of diabetes and hypertension, increased levels of Very-Low-Density Lipoprotein (VLDL)-triglycerides, and Low-Density Lipoprotein (LDL) cholesterol, and decreased levels of high-density lipoprotein cholesterol, all of which are risk factors for the development of vascular disease[45]. Obesity as a characteristic feature of PCOS, and its possible connection with the GMB can be hypothesized. Variations in the microbial genome colonizing our body may have a role in pathogenesis of obesity due to its direct interaction with environmental factors, and microbial differences can classify individuals as lean or obese based on the presence of phyla Firmicutes, Bacteroidetes and Actinobacteria ratios[46]. Alterations in the permeability of intestinal flora lead to endotoxemia within the bloodstream. Diabetes and obesity, characterized by chronic low-grade inflammation and IR, lead to possible inflammatory triggers by the GMB due to LPS where metabolic endotoxemia dysregulates the inflammatory tone and triggers body weight gain or obesity and diabetes[47].

Obesity and metabolic comorbidities are becoming prevalent health issues, and EDCs alter endocrine function to produce negative consequences in health. Hormone-dependent metabolic systems and brain function are both affected by EDCs. According to laboratory and human studies, human chemical contamination may play a role in the obesity epidemic. Chemical exposures may increase the risk of obesity by altering the differentiation

of adipocytes[48]. Alteration of gut bacteria is strongly linked to changes in the microbiota metabolites. These changes increase the risk of tissue dysfunctions that might lead to obesity, IR, and CVD. Studies conducted by Fan *et al.*[49] indicated that an increase in the risk of obesity and metabolic syndrome is linked to exposure of low doses of di-(2-ethylhexyl) phthalate during the early stages of life. Studies in rodents (ICR, C57Bl/6, and CD-1 mice and Wistar rats) found that pesticides (carbendazim, chlorpyrifos and organophosphorus pesticides) cause microbiota dysbiosis and inflammation, altering lipid metabolism and triggering obesity in exposed rodents[50]. Increased levels of carbendazim exposure in mice lead to disturbed lipid metabolism and gut dysbiosis, indicating that this EDC potentially interferes with the microbiota environment, rendering inflammation and triggering obesity[51]. Studies have identified associations between obesity and gut-microbiome energy-harvesting capabilities in obese mice, thereby providing a basis to investigate similar interactions between human obese individuals and their microbial communities, thus acting as a plausible mediator and a therapeutic approach to target obesity in general, and PCOS-induced obesity especially.

4.2 PCOS-GMB: Insulin Resistance and Type-2 Diabetes

IR manifested in a minimum of 50% of PCOS is due to excessive phosphorylation of serine residues of the insulin receptor, thus decreasing the signal transduction pathway. Genetic predisposition and inherent weight problems add to the metabolic IR in women affected with PCOS[52]. Oxidative stress, characterized by the presence of Reactive Oxygen Species (ROS), occurs due to the imbalance in the scavenging mechanism by antioxidant enzymes. An increase in the levels of oxidative stress is manifested in PCOS women, and the subsequent ROS release from MNCs might be a trigger for IR[53]. The ROS interferes with the normal glucose uptake metabolism in the muscle cells and cells of the adipose tissue by decreasing the production of insulin required for glucose uptake thereby contributing to IR and hyperinsulinemia making the individual susceptible to T2D. Studies suggest that the defects in insulin activity and related pathways play a critical role in the pathogenesis of the syndrome. IR is not shown in all women with typical PCOS, thus supporting the hypothesis of a genetic predisposition specific to PCOS that might be discovered by the event of IR and countervailing hyperinsulinemia[52]. Hyperactivity of the HPA axis develops metabolic syndromes as manifested in PCOS, and studies have indicated that insulin regulates adrenal steroidogenesis via the activity of steroidogenic factor 1[54] and, in doing so, create a bridge between the HPA and Gut-Brain axis in PCOS. As previously explained, the intestinal microbiota is associated with metabolic abnormalities such as IR and an ensuing condition of T2D. Low-grade inflammation of the visceral adipose tissue links obesity and IR, and a similar condition caused by an increase in gram-negative bacterial strains, is observed in patients with T2D. Reexploring the condition of metabolic endotoxemia caused by the gut microbiota rendered an impaired glucose metabolism due to LPS circulation in mice, requiring a relevance to study this impaired glucose metabolism and IR in humans.

EDCs appear to play an important role in the etiology of diabetes and metabolic disorders according to a growing body of evidence. EDCs have been linked to hyperglycemia, glucose intolerance, and insulin resistance in several experimental and epidemiological studies[55]. Interactions with the aryl hydrocarbon receptor (AhR) and nuclear hormone receptors, including estrogen receptors, alteration of ERK/Akt signaling pathways, induction of oxidative and nitrosative stress, pancreatitis, and dysregulated hepatic metabolism are among the proposed mechanisms of action of EDCs. It has been found that exposure to PCB126 (1 mmol/kg) disrupts the gut microbiota and host metabolism[56]. Although mounting evidence suggests that these molecular mechanisms underpin EDCs' effects on diabetes development, a comprehensive understanding of their mechanism of action is still missing. The GMB as a target to treat T2D was explored adopting a variety of sequencing, studying metabolomics and microbiome profiling to subjects with T2D to identify differences in the gut microbiota. Discerning of the precise microbiome composition to determine the exact metabolic parameters is imperative to comprehend the interrelationship between the EDCs on the GMB and the endocrine-immune network[57]. Establishment of this mechanism utilizing advancements in technologies can provide the GMB as a successful therapeutic target for EDC causing IR and T2D in PCOS women.

4.3 PCOS-GMB: Cardiovascular Disease

Metabolic disturbances due to PCOS can be manifested as cardiometabolic disorders. These disorders consist of a spectrum of metabolic diseases including IR, impaired glucose tolerance, dyslipidemia, adiposity, hypertension and cardiovascular CVD. The risk factors for vascular diseases are accelerated by existing conditions of obesity combined with increased levels of VLDL triglycerides, LDLc and decreased levels of HDLc in PCOS women. Women with PCOS constitute a distinctive group with an increased risk for developing CHD due to a high frequency of PCOS women diagnosed with T2D[58]. Insulin as a regulator of metabolism confers metabolic irregularities in the state of IR often inducing possible developments of CVD substantiating claims of PCOS accelerating cardiometabolic disorders. Systemic lipid metabolism is altered by IR, causing dyslipidemia characterized by high levels of plasma triglycerides, low levels of high-density lipoprotein and appearance of small dense low-density lipoproteins[59]. Adipose tissue IR can also be used as an indicator for cardiometabolic disorders caused to the IR mediated release of Free Fatty Acids (FFA) from adipocytes[60]. Studies connecting visceral and subcutaneous abdominal fat thickness with cardiometabolic risk factors indicated a positive correlation between visceral adipose tissue and CVD along with testosterone in PCOS patients, indicating the effect of IR, glucose metabolism and hormonal parameters in PCOS, contributing to CVD risk[61]. A plausible mechanism between the action of the GMB in the incidence of CVD is also hypothesized, establishing a PCOS-GMB link in the manifestation and control of CVD. Bacterial metabolites possibly regulate pathophysiology of certain diseases causing disruption and tenable cardiometabolic risks. Cholesterol, documented as a risk factor for CVD and most commonly coronary artery disease, is balanced and controlled by the gut lumen. The direct influence of the gut on metabolic disorders can be via metabolites and indirectly via immune system modulation. Bile acid production can be deregulated by altering the absorption of fats and nutrients and dysregulate lipid, energy and glucose metabolism merely by fluctuations in the ratio of specific microbiota. Conceivable mechanisms indicate that the microbiota which modulate bile acid ratios leading to unbalanced ratios, could lead to reduced secondary bile acids and subsequently increasing cholesterol and CVD risk[59]. Genetic markers of dyslipidemia rendering high CVD risks are influenced by the GMB; however, non-genetic markers can similarly indirectly influence the environment of the gut rendering individuals susceptible to a metabolic imbalance[62]. It has been shown that exposure to arsenic, an EDC, altered the gut bacteria and microbial metabolites, based on which it was hypothesized that these alterations can potentially increase the risk of tissue dysfunctions that might lead to cardiovascular disease. Further studies are required to link EDCs to CVDs. However, cardiometabolic disorders and/or fatal CVDs have been established in cases of PCOS, providing a plethora of targets in association with the GMB for its alleviation and manageable personalized treatments.

4.4 PCOS-GMB: Hyperplasia and Gynecological Cancer

A plethora of metabolic conditions are manifested in PCOS women. However, adverse long-term metabolic and endometrial consequences of PCOS have been documented in two women in the form of endometrioid carcinoma and endometrial atypical hyperplasia[63]. The adverse effects of dysregulation of steroidogenesis and hyperandrogenism are manifested as hyperplasia. PCOS influences dietary intake and metabolism, providing a plausible link between BMI and obesity as a mediator in cancer associations. The epidemiology of endometrial cancers emphasizes uterine cancer to be the most common gynecological cancer the growth of which is nourished by the perpetuated conditions of increased estrogen and decreased progesterone, which is prevalent in conditions of PCOS, obesity and diabetes. Chronic anovulation, characteristic of PCOS, exposes uterus to prolonged estrogen stimulation uncontested by progesterone, increases the risk of endometrial hyperplasia and carcinoma[64]. Among women diagnosed of PCOS in their reproductive ages, the risk of endometrial cancer due to PCOS-induced endometrial hyperplasia is higher than in non-PCOS women[65] thus vindicating the requirement to keep this condition under control by targeting adrenal and ovarian steroidogenesis. As previously mentioned, abnormal steroidogenesis owes to environmental, genetic and microbiota cues. The CYP gene is responsible for androgen production and hyperandrogenism in PCOS women and causes dysregulation of enzymes concerned with steroid synthesis and imparts hyperplasia of ovarian thecal cells. The uterine microbiome was studied to

be a possible contributor towards the development of endometrial cancer owing to the association between microbiome shift within the vagina, cervix, Fallopian tubes, and ovaries in hyperplasia and cancer cases[66]. Dysbiosis of gut microbiota caused due to prenatal androgen exposure can lead to further metabolic abnormalities within the offspring as well as an increase in susceptibility towards development of hyperandrogenemia and PCOS-induced hyperplasia[67]. Endometrial dysfunction can induce infertility in PCOS women and it was observed that metformin in combination with oral contraceptives would reverse endometrial atypical hyperplasia in obese, progestin-resistant women with PCOS[63]. EDCs, on the other hand, can impair the function of the endocrine system through a variety of mechanisms. EDCs interact with Estrogen Receptors (ERs) or alter estrogen signaling pathways and cause adverse effects within the endocrine system[68]. The phenolic structure of BPA also aids in its interaction with ERs and associated signaling pathways[69]. While the influence of the GMB on PCOS-associated cancers has been highlighted, the data on the precise action of EDCs in the pathogenesis of uterine hyperplasia and cancers is not yet sufficient. The effects of EDCs on the endometrium and the plausible association between endometrial disorders and BPA exposure have been demonstrated[70]. The phytoestrogen daidzein, an EDC, was found to cause endometrial growth and hyperplasia in ovariectomized rats[71] consistent with the data indicating a higher incidence of endometrial hyperplasia in women consuming soy extracts[72]. EDCs such as BPA, nonylphenol, octylphenol, methoxychlor, benzophenone-1, etc., stimulate the proliferation of ER-positive BG-1 ovarian cancer cells via estrogen signaling pathway[73,74]. Studies associating estrogenic EDC exposure with the risk of gynecological cancers are thus crucial, and studying the GMB in women with PCOS exposed to EDCs can aid in understanding the interconnected networks. Thus, treatment strategies involving utilization of GMB towards altering steroidogenesis, targeting diabetes, IR and obesity can contribute to a decreased liability of endometrial thickening and in rare cases endometrial or uterine cancer, decrease of cases of infertility and increase of conception rates in PCOS women.

5. Conclusion

PCOS, a composite syndrome, is influenced by a torrent of genetic factors, and internal and external environmental conditions contributing to its pathogenesis as an endocrine and metabolic dysfunction disorder. Genes associated with steroid synthesis pathway, carbohydrate and lipid metabolism, immune and inflammatory responses related to PCOS provide a plausible correlation between metabolic factors and the influence of the GMB in the manifestation of chronic low-grade inflammation as a contributor to PCOS. Maintenance of the microbes ensures a healthy gut which subsequently controls adjacent homeostatic functions. Fundamental to developing novel treatment opportunities for PCOS and affiliated disorders of this syndrome, is understanding the role of the gut-brain axis between the CNS and the GI tract and its role in the development and maintenance of the environs of the gut. Interlinking the GMB with PCOS provides a wide range of opportunities to study the syndrome and simultaneously aids in eliminating increased risks of infertility, endometrial hyperplasia and cancer, abnormal glucose metabolism, dyslipidemia, obstructive sleep apnea and even psychological conditions such as depression and anxiety. Metabolic diseases such as obesity and T2D are becoming more common around the world. Microbial dysbiosis and the induction of xenobiotic pathways, as well as associated genes, enzymes, and metabolites involved in EDC metabolism, are all induced by EDC exposure related to food intake. Clinical studies linking EDC exposure, PCOS and the development of female gender-related comorbidities and malignancies are required, but the level and timing of EDC exposure must also be considered. The remediation of EDC-induced changes in the gut microbiome might represent an alternative for the treatment and prevention of metabolic comorbidities associated with PCOS. Monitoring and conservation of the gut from foetal to adult stages with focus on altering the endocrine, immune and metabolic networks associated with PCOS can prove to be a plausible technique to handle EDC- associated PCOS in the near future.

6. Declaration

The authors have no conflict of interest to declare.

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Nil.

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