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Original Article

Integration of cf-mtDNA Liquid Biopsy and Quercetin-Inositol (Quercitol) Nanoliposomes: A Mitochondria-Based Diagnostic and Therapeutic Approach for Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a complex endocrine-metabolic disorder with limited conventional diagnostic criteria and heterogeneous symptoms, especially in adolescents, contributing to the frequent underdiagnosis of PCOS. Recent studies have shown the role of mitochondrial dysfunction and increased cell-free mitochondrial DNA (cf-mtDNA) in the pathogenesis of PCOS, which has the potential to be used as a non-invasive biomarker based on liquid biopsy. This study aims to review the role in cf-mtDNA to diagnose and the potential of quercetin and inositol as mitochondria-based therapies. The writing method is a systematic literature review of 40 articles between 2016 and 2026 from PubMed and Google Scholar relevant to the development of research on cf-mtDNA, quercetin, and inositol in PCOS. The findings indicate that cf-mtDNA levels increase in PCOS patients and correlate with oxidative stress and insulin resistance, thus having the potential to become a non-invasive diagnostic indicator. Therapeutically, quercetin exhibits antioxidant and protective effects on mitochondria, while inositol improves insulin sensitivity and ovulation function. The combination of both compounds (quercitol) in a nanoliposome delivery system modified with triphenylphosphonium (TPP+) enhances targeting to mitochondria and biological efficacy. It can be concluded that the theranostic approach integrating cf-mtDNA biomarkers and nanoliposomal quercetin therapy presents an innovative, precise, and non-invasive strategy for personalized PCOS management.

Keywords: *biomarker, cf-mtDNA, inositol, kuersetin, nanoliposom, PCOS*

ABSTRAK

Sindrom Polikistik Ovarium atau Polycystic Ovary Syndrome (PCOS) merupakan gangguan endokrin–metabolik kompleks dengan keterbatasan kriteria diagnostik konvensional serta heterogenitas gejala, sehingga menyebabkan PCOS underdiagnosed. Studi terkini menunjukkan peran

disfungsi mitokondria dan peningkatan cell-free mitochondrial DNA (cf-mtDNA) dalam patogenesis PCOS yang berpotensi sebagai biomarker non-invasif berbasis biopsi cair. Kajian ini bertujuan untuk meninjau peran cf-mtDNA untuk diagnosis serta potensi kuersetin dan inositol sebagai terapi berbasis mitokondria. Metode penulisan berupa literature review sistematis terhadap 40 artikel tahun 2016–2026 dari PubMed dan Google Scholar yang relevan dengan perkembangan penelitian cf-mtDNA, kuersetin, dan inositol pada PCOS. Hasil kajian menunjukkan bahwa kadar cf-mtDNA meningkat pada pasien PCOS dan berkorelasi dengan stres oksidatif serta resistensi insulin, sehingga berpotensi menjadi indikator diagnostik non-invasif. Secara terapeutik, kuersetin menunjukkan efek antioksidan dan protektif terhadap mitokondria, sedangkan inositol memperbaiki sensitivitas insulin dan fungsi ovulasi. Kombinasi keduanya (kuersitol) dalam sistem penghantar nanoliposom yang dimodifikasi dengan triphenylphosphonium (TPP+) meningkatkan penargetan ke mitokondria serta efikasi biologis. Dapat disimpulkan bahwa pendekatan teranostik yang mengintegrasikan biomarker cf-mtDNA dan terapi kuersitol nanoliposomal menghadirkan strategi inovatif yang presisi dan non-invasif dalam manajemen personal PCOS.

Keywords: *biomarker, cf-mtDNA, inositol, kuersetin, nanoliposom, PCOS*

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a complex reproduction disorder centered on the ovaries and commonly occurs in women of reproductive age (18–39 years).[1] The diagnosis of PCOS is based on clinical manifestations such as irregular menstrual cycles, acne, excessive hair growth (hirsutism), obesity, and high insulin levels. Furthermore, the diagnosis is established through ultrasonography (USG), which identifies an ovarian volume of more than 10 mL and the presence of at least ten small follicles (2–9 mm) in either ovary.[2]

Epidemiologically, the World Health Organization (WHO) reports that the prevalence of PCOS among women of reproductive age ranges from 8–13%, with more than half of cases remaining undiagnosed.[3] In line with this, a global systematic screening study using the National Institutes of Health (NIH) diagnostic criteria estimates that approximately 4–10% of women of reproductive age have PCOS.[2] Meanwhile, data from Indonesia indicate that the prevalence of PCOS among adolescent girls in Jakarta schools is 6.5% based on the Rotterdam diagnostic criteria.[4] Diagnostic delays commonly occur because PCOS symptoms are heterogeneous and are often only recognized once the condition has progressed further and is affecting the patient's quality of life.

The heterogeneous nature of PCOS symptoms makes it difficult to establish a diagnosis based on a single clinical manifestation and can lead to ambiguity due to similarities with other conditions, such as Non-Classic Congenital Adrenal Hyperplasia (NCAH); therefore, specific laboratory tests are required to rule out these differential diagnoses. In addition to the heterogeneity of symptoms, PCOS is also divided into four phenotypes, A, B, C, and D, each characterized by a distinct combination of three symptoms. Furthermore, the assessment of polycystic ovarian morphology (PCOM) during diagnosis may yield variable results, as it depends on the type of transvaginal ultrasound equipment and the operator's skill. Variations in PCOS manifestations across age groups, particularly in adolescents with suspected PCOS, further add to the complexity of the diagnostic process regarding PCOM.[5]

Given the challenges in diagnosing PCOS, an objective and practical approach applicable across all age groups is required. A body of evidence suggests that mitochondrial dysfunction plays a key role in the pathophysiology of PCOS, which is characterized by impaired mitochondrial biogenesis and function, as well as increased oxidative stress.[6] This condition is associated with changes in the levels and release of cell-free mitochondrial DNA (cf-mtDNA), which holds potential for development as a noninvasive biomarker.[7] The use of cf-mtDNA via liquid biopsy offers diagnostic advantages due to its noninvasive nature and applicability across various age groups, including adolescents for whom conventional diagnostic methods are limited.[7] Beyond diagnostic aspects, therapeutic approaches targeting mitochondrial dysfunction, such as the use of quercetin with its antioxidant properties and inositol, which plays a role in metabolic therapy, show potential as adjuvant therapies in the management of PCOS.[8] Therefore, this literature review aims to examine the role of mitochondrial dysfunction and cf-mtDNA as the basis for a diagnostic approach and to evaluate the potential of quercetin and

inositol as mitochondrial-based therapeutic strategies for PCOS.

METHODS

The research method employed was a literature review. The author used the following keyword combinations: "cf-mtDNA", "Biomarker," "Liquid," Biopsy," "Quercetin," "Inositol," "Nanoliposome," "Mitochondria," and "PCOS" along with their synonyms using the Boolean logic operators "AND" and "OR" to search for scientific articles in the PubMed and Google Scholar databases. The author established inclusion and exclusion criteria based on the topic of discussion. The inclusion criteria used were that the studies were published within the last 10 years (2016–2026). Meanwhile, the exclusion criteria applied were that the studies were classified as gray literature or that the full text was inaccessible. The author used 40 articles as references and organized them systematically to present the entire content of the literature review.

REVIEW

Pathophysiology and Diagnostic Criteria for PCOS as an Endocrine-Metabolic Disorder

Genetic and environmental factors play a role in the disruption of the hypothalamic–pituitary–ovarian (HPO) axis, which triggers various clinical manifestations in PCOS, leading to its classification as a multifactorial syndrome. One of the early processes that plays a key role in the pathogenesis of PCOS is increased ovarian steroidogenesis mediated by cytochrome P450 (CYP) genes, resulting in excessive androgen production. This condition of hyperandrogenism indirectly contributes to the development of insulin resistance and increased oxidative stress, which underlie the clinical manifestations in patients with PCOS.[2]

Hyperandrogenism in PCOS

Physiologically, androgens are present in women's bodies in small amounts to maintain sexual function, muscle mass, and bone density.[1] The condition becomes pathological when these hormones are present in excess, which occurs in 70–90% of women with PCOS.[2,9] The clinical manifestations commonly resulting from hyperandrogenism include hirsutism, acne, and alopecia.[10]

This process begins in the pituitary gland, which is highly sensitive to Gonadotropin-Releasing Hormone (GnRH), leading to the production of Luteinizing Hormone (LH) at levels far higher than normal. Excess LH stimulates the growth and proliferation of ovarian theca cells, resulting in hyperplasia. This abnormality activates the CYP11A1 gene, which encodes the cholesterol-desmolase enzyme for the conversion of cholesterol into pregnenolone. Subsequently, the enzymes 17α-hydroxylase and 17,20-lyase, encoded by the CYP17A1 gene, convert pregnenolone into dehydroepiandrosterone (DHEA) or weak androgens. Meanwhile, the enzyme 3β-hydroxysteroid dehydrogenase catalyzes the conversion of DHEA into androstenedione and of pregnenolone into progesterone, which play a crucial role in the formation of androgens and progesterone.[9]

Ovulatory Dysfunction (Anovulation)

In contrast to the increased LH levels, due to disruption of the HHO axis, the production of follicle-stimulating hormone (FSH) to stimulate follicular maturation decreases, causing the follicles to stop developing (follicular arrest). When a dominant follicle does not form, there is no egg and no ovulation.[5] This ovulatory dysfunction can lead to amenorrhea, abnormal uterine bleeding, and infertility as clinical manifestations in PCOS patients. A symptom frequently observed in 40% of PCOS patients is oligo-amenorrhea or menstrual cycle disorders characterized by infrequent and irregular periods, which may include the complete absence of menstruation for ≥ 3 consecutive months (amenorrhea) or intervals between periods >35 days (oligomenorrhea).[1] However, oligomenorrhea is difficult to detect in adolescents because ovulatory dysfunction and irregular menstrual cycles are considered normal during the transition of puberty. Therefore, international PCOS guidelines define irregular menstrual cycles in adolescents based on gynecological age, meaning irregularities are still considered normal during the first year post-menarche. In the 1–3 years following menarche, cycles <21 or >45 days are considered abnormal, whereas after >3 years following menarche, cycles <21 or >35 days or <8 cycles per year are considered irregular. An interval of >90 days between cycles after the first year post-menarche, as well as primary amenorrhea up to age 15 or >3 years post-menarche, are also categorized as abnormal.[5]

Morphology of Polycystic Ovaries (PCOM)

Clinical manifestations result from the arrest of follicular maturation in the ovaries. Normally, small-to-medium-sized follicular granulosa cells produce Anti-Müllerian hormone (AMH) to regulate follicular development in the ovaries, and AMH levels decrease in the dominant follicle. In PCOS, a dominant follicle does not form, leading to an accumulation of small-to-medium-sized follicles that trigger a fourfold increase in AMH production and a reduced follicular response to FSH. Therefore, transvaginal ultrasound examination in patients with PCOS reveals the characteristic appearance of polycystic ovaries.[1,5] However, there are limitations regarding the diagnostic accuracy of transvaginal ultrasound due to various factors, such as age, ethnic background, body mass index (BMI), and operator variability, making PCOS difficult to detect in adolescents.[5]

The Rotterdam Diagnostic Criteria and the PCOS Phenotype

In 2003 in Rotterdam, a meeting was held, initiated by the European Society for Human Reproduction and

Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). Through this meeting, PCOM was added as one of the diagnostic criteria, which subsequently became known as the Rotterdam criteria and are still widely used today.[10]

In 2023, the Rotterdam diagnostic criteria were revised to state that a diagnosis of PCOS can be established if two of the three clinical manifestations, hyperandrogenism, oligo/amenorrhea, and PCOM, are present, with PCOM specifically indicated for adults. Additionally, these clinical manifestations must be ruled out by other secondary factors through a standardized diagnostic algorithm (Figure 1).[5]

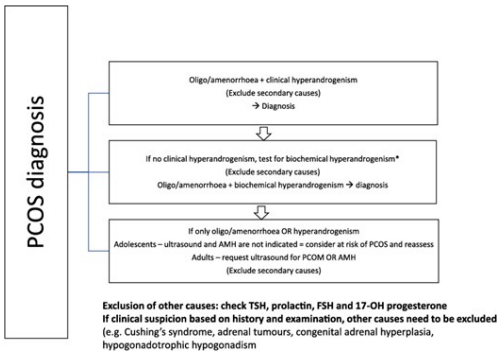

Figure 1. *PCOS Diagnostic Algorithm.[5]*

Based on these three clinical manifestations, which serve as diagnostic criteria, the medical community has identified four phenotypes representing variations in the clinical presentation of PCOS. The phenotypes are classified as A through D, ranging from the most severe to the mildest (Figure 2).[2,5,9]

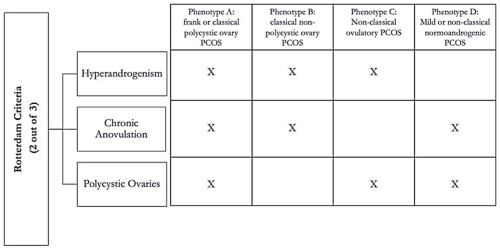

Figure 2. *Phenotypic Variations of PCOS.[9]*

Mitochondrial Dysfunction in the Pathogenesis of PCOS

In addition to diagnostic approaches based on clinical manifestations, various studies have sought to elucidate the pathophysiological mechanisms of PCOS at the cellular and molecular levels. One mechanism that has garnered attention in the pathogenesis of PCOS is mitochondrial dysfunction as a link between endocrine and metabolic disorders.

Mitochondria as an Early Target of Endocrine-Metabolic Disorders in PCOS

regulating mitochondrial dynamics, and the accumulation of reactive oxygen species (ROS), all of which contribute to energy and metabolic disturbances in the early stages of PCOS before other clinical manifestations become apparent (Figure 3).[6] Clinical and experimental studies also confirm that these changes emerge as early as the phase of hormonal and metabolic disturbances in PCOS, making mitochondria an early target of endocrine-metabolic dysfunction in PCOS.[11]

The pathophysiological process of PCOS begins with a disruption of the endocrine system, specifically a hormonal imbalance that leads to hyperandrogenism. A state of excess androgens in the body affects cellular activity in the ovaries (including granulosa cells and oocytes) by increasing cellular energy requirements and altering the expression of genes involved in the regulation of mitochondrial metabolism. In a physiological context, mitochondria are central organelles in cellular energy regulation through ATP production, the regulation of lipid and glucose metabolism, and play a crucial role in metabolic and reproductive tissue function. Mitochondrial dysfunction in ovarian cells includes impaired biogenesis, reduced oxidative phosphorylation capacity, ultrastructural changes, imbalances in proteins

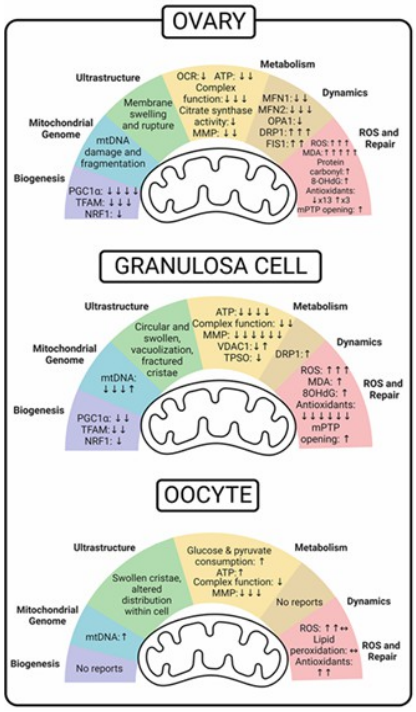

Figure 3. *Mitochondrial Dysfunction in PCOS.[6]*

The Relationship Between Hormonal Imbalance, Insulin Resistance, and Mitochondrial Dysfunction

Mitochondrial functional changes resulting from hormonal imbalances indicate that mitochondria are sensitive to the hormonal changes that occur in PCOS. High androgen levels increase lipolysis in adipose tissue,

leading to elevated levels of free fatty acids in the circulation. This increase in free fatty acids disrupts insulin signaling pathways in peripheral tissues, thereby reducing insulin sensitivity. Furthermore, insulin resistance exacerbates PCOS because cells become less efficient at taking up glucose and tend to rely on fatty acid oxidation to seek alternative energy sources. This oxidative inefficiency can disrupt the mitochondrial electron transport chain and reduce ATP production, thereby worsening mitochondrial dysfunction and insulin resistance itself, as it requires electrons and generates more ROS. Therefore, a reciprocal relationship forms between insulin resistance, lipid metabolic signaling, and mitochondrial dysfunction as one of the mechanisms of hormonal and metabolic dysfunction in PCOS.[12]

Oxidative Stress (ROS) and the Vicious Cycle of Mitochondrial Dysfunction

Under normal conditions, ROS are byproducts of oxidative phosphorylation (OXPHOS) that can be neutralized by the body's natural antioxidants. However, PCOS with mitochondrial dysfunction disrupts the OXPHOS process and increases electron leakage, leading to elevated ROS levels. Meanwhile, individuals with PCOS often have an inadequate antioxidant system, leading to oxidative stress that damages mitochondrial DNA (mtDNA), respiratory proteins, and mitochondrial membranes.[6] This mitochondrial damage caused by ROS contributes to reduced ATP production and interacts with insulin resistance, creating a vicious cycle.[11]

cf-mtDNA as a Liquid Biopsy-Based Biomarker

Given the role of mitochondrial dysfunction and oxidative stress in the pathophysiology of PCOS, as well as the limitations of current conventional procedures, there is a need for non-invasive biomarkers capable of reflecting these changes systemically. In this context, cell-free mitochondrial DNA (cf-mtDNA) has the potential to serve as a liquid biopsy-based biomarker, as its release into the circulation is associated with mitochondrial damage and metabolic disturbances.

cf-mtDNA and the Concept of Liquid Biopsy

Cell-free mitochondrial DNA (cf-mtDNA) consists of mitochondrial DNA fragments released into body fluids, such as plasma, serum, or follicular fluid, as a result of cellular stress, mitochondrial dysfunction, or cell death via apoptosis or necrosis. Biomarkers, such as cf-mtDNA, can be measured non-invasively through body fluids to reflect the pathological condition of specific tissues. The concept of liquid biopsy has been extensively developed, particularly in the field of oncology, where cf-mtDNA has been shown to correlate with disease status and reflect the degree of mitochondrial damage as well as cellular metabolic activity.[13]

In PCOS, mitochondrial dysfunction occurring from the early stages has the potential to trigger the release of mtDNA into the extracellular environment. This is supported by findings that cf-mtDNA levels in the follicular fluid of PCOS patients differ significantly from those of women without PCOS and are associated with changes in mitochondrial gene expression in granulosa cells.[7] Thus, cf-mtDNA is biologically relevant to be considered as a liquid biopsy-based biomarker reflecting mitochondrial and metabolic disturbances in PCOS.

cf-mtDNA as an Indicator of Mitochondrial Dysfunction in PCOS

Under conditions of oxidative stress, mtDNA becomes fragmented and released, acting as damage-associated molecular patterns (DAMPs) and activating inflammatory pathways.[6] This links mitochondrial dysfunction to both systemic and local inflammatory responses in the ovaries. In PCOS, cf-mtDNA levels have been associated as markers of oxidative stress and inflammation in follicular fluid, reflecting disturbances in the ovarian microenvironment.[7] Furthermore, studies indicate that mitochondrial dysfunction in PCOS contributes to insulin resistance and metabolic disorders, thereby indirectly reinforcing the link between mitochondrial damage and the release of mtDNA into the circulation.[14] Therefore, cf-mtDNA has the potential to be used as a non-invasive indicator reflecting mitochondrial dysfunction and metabolic disturbances consistent with the primary pathophysiology of PCOS in the form of fluid biopsy.

The Potential of Quercetin and Inositol as Therapeutic Agents for PCOS Management

Quercetin

Quercetin is a flavonoid polyphenol compound that possesses antioxidant and anti-inflammatory properties, as well as various other biological effects. This compound is widely found in fruits, green leafy vegetables, and various types of grains. Quercetin has been extensively studied as a potential therapeutic candidate for women with PCOS. In vitro studies indicate that quercetin can alleviate oxidative stress and apoptosis in ovarian cells (Appendix 1). Previous research has shown that quercetin effectively reduces serum testosterone levels, luteinizing hormone (LH), fasting insulin, and blood lipid levels in both human patients and animal models of PCOS (Appendix 2). Animal experiments also demonstrate that quercetin increases the number of preantral, antral, and pre-ovulatory follicles, as well as the number of corpus luteum, while reducing the number of atretic follicles and eliminating cyst formation in PCOS mouse models. Its potential mechanisms of action include improvement of insulin resistance, anti-inflammatory effects, mitigation of

oxidative stress, anti-apoptosis, and downregulation of CNP/NPR2 expression (Figure 4).[15]

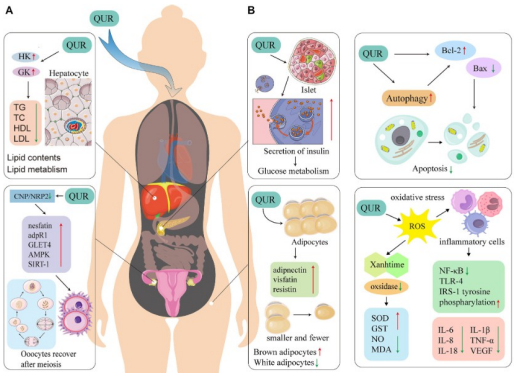

Figure 4. *Potential Targets for Quercetin Therapy in PCOS.[15]*

Given that the pathogenesis of PCOS is closely linked to mitochondrial dysfunction, quercetin plays a significant role in regulating the function of these organelles. A study by Ho et al. showed that in H_2O_2-induced SH-SY5Y cells, quercetin not only stimulates the expression of mitochondria-related proteins, such as SIRT1, PGC-1α, and TFAM, but also activates mitochondrial biogenesis. Additionally, quercetin increases ADAM10 expression while suppressing the production of reactive oxygen species (ROS), apoptosis, the expression of the β-site amyloid precursor protein cleaving enzyme 1 (BACE1), and Aβ accumulation. Thus, the efficacy of quercetin is closely associated with increased expression of mitochondrial biogenesis proteins and reduced damage caused by oxidative stress. At the ovarian cellular level, quercetin has been shown to restore mitochondrial membrane potential, increase ATP production, and reduce mitochondrial DNA damage in granulosa cells and oocytes. Quercetin's dual role as an antioxidant and a mitochondria-protective agent highlights its potential to address reproductive and metabolic dysfunction simultaneously, thereby strengthening its position as a multi-target agent in PCOS therapy.[16,17]

Inositol

Inositol, a B-complex vitamin with antioxidant properties, along with its stereoisomers—myo-inositol and D-chiro-inositol—has been studied as a treatment for PCOS. Based on a meta-analysis by Sene et al., the consumption of myo-inositol or D-chiro-inositol has a significant impact on clinical pregnancy rates, increases the number of high-quality embryos, and reduces the average antral follicle count (AFC) and anti-Müllerian hormone (AMH) levels in women with PCOS. This improvement in embryo quality may be attributed to the effects of myo-inositol, which has been shown to modulate gene expression in granulosa cells and enhance oocyte and embryo quality in women with PCOS.[18] A study by Yuan et al. demonstrated that myo-inositol can reduce fasting serum insulin levels through insulin-related metabolic pathways, thereby improving the hyperinsulinemia associated with insulin resistance. On the other hand, D-chiro-inositol supplementation can lower serum insulin and improve insulin sensitivity in PCOS patients with impaired glucose tolerance, ultimately improving ovulatory function.[19]

The Efficacy of the Combination of Quercetin and Inositol (Quercitol)

A comprehensive scientific review by Yadunandan et al. confirms that inositol and quercetin have distinct yet synergistic mechanisms of action in the treatment of PCOS. The study states that inositol primarily focuses on improving insulin resistance and ovulatory dysfunction, while the antioxidant properties of quercetin play a crucial role in maintaining hormonal balance and supporting the process of folliculogenesis. Furthermore, the results of this analysis indicate that the combination of inositol and quercetin is capable of improving insulin sensitivity, suppressing hyperandrogenism, and addressing oxidative stress more effectively. The integration of these two compounds is viewed as a promising strategy for producing superior therapeutic benefits compared to their use individually.[8]

Nanoliposomes as a Strategy to Enhance the Efficacy of Quercetin

The use of nanoliposomes as a delivery system is a crucial strategy for overcoming the various pharmacokinetic challenges associated with quercetin. Although this compound has great therapeutic potential, quercetin degrades rapidly in the gastrointestinal tract and undergoes extensive metabolism in the liver before reaching the target tissues. This results in low effective plasma concentrations, thereby limiting its therapeutic effects on PCOS. Research data indicate that the estimated bioavailability of quercetin is only 44.8%, suggesting that the majority of the compound is not optimally absorbed.[15]

To overcome these barriers, a nanoliposome encapsulation approach has been developed to enhance treatment efficacy, particularly when combined with other compounds such as inositol. The structure of nanoliposomes, consisting of a phospholipid bilayer and an aqueous core, allows for a combination strategy between quercetin and inositol within a single delivery system (Figure 5).[20,21]

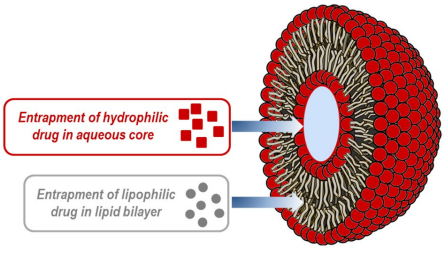

Figure 5. ***Liposome Structure.[22]***

In this formulation, the hydrophobic quercetin is protected within the lipid bilayer from oxidative degradation, while the hydrophilic inositol is trapped within the aqueous core of the nanoliposomes. This integration not only enhances the dispersibility of both substances in the aqueous phase but also enables controlled release in the intestine. A study by Liu et al. showed that a nanoliposome formulation with an average size of 134 nm and an encapsulation efficiency of 63.73% exhibits high stability (zeta potential -37.5 mV), which significantly preserves the biological activity of the active compounds within biological systems. Thus, the quercetin-inositol combination in a nanoliposome system offers stronger therapeutic synergy to improve the metabolic and hormonal profiles in patients with PCOS.[23]

Nanoliposome Modification: Mitochondrial Targeting with TPP+

The use of quercetin nanoliposomes that target mitochondria is an innovative strategy for addressing the metabolic dysfunction and oxidative stress characteristic of PCOS. This strategy specifically employs triphenylphosphonium (TPP+), a lipophilic cation with a delocalized positive charge, as a highly efficient targeting agent. This targeting mechanism is based on the electrostatic attraction between the positively charged TPP+ groups and the highly negative mitochondrial membrane potential (ΔΨm), which ranges from -150 to -180 mV. This large potential difference allows the nanoliposomes to penetrate the complex mitochondrial double membrane and accumulate selectively within the organelle's matrix at concentrations 100–500 times higher than in the cytoplasm (Figure 6). Direct intervention at the mitochondrial level is capable of reducing free radical production, improving cellular respiration, and restoring impaired mitochondrial biogenesis in PCOS patients.[24,25,26]

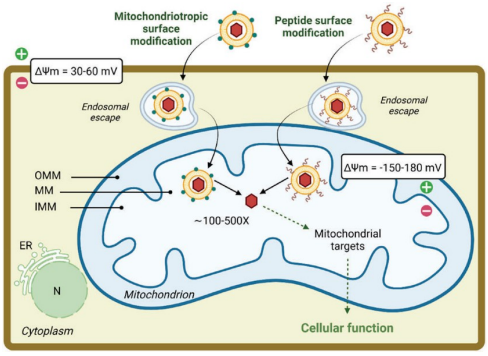

Figure 6. *Schematic of Liposome Targeting to Mitochondria.[24]*

Theranostic Integration: cf-mtDNA and Nanoliposomal Quercetin in the Management of PCOS

The theranostic approach to PCOS integrates the role of cf-mtDNA as a liquid biopsy biomarker with nanoliposomal quercetin as a precision-targeted therapy. Pathophysiologically, chronic oxidative stress in PCOS patients causes mitochondrial membrane damage, leading to the release of DNA fragments into the bloodstream (cf-mtDNA). These cf-mtDNA levels serve as a diagnostic indicator to non-invasively measure the degree of cellular dysfunction. As a therapeutic solution, a combination of quercetin (an antioxidant) and inositol (a metabolic regulator) is encapsulated in a nanoliposome system modified with TPP+ molecules. This modification ensures that the active substances are specifically delivered into mitochondria experiencing stress. The success of this intervention is then revalidated through cf-mtDNA monitoring. A decrease in cf-mtDNA levels in the blood serves as objective evidence of improved mitochondrial integrity and the success of PCOS therapy. This theranostic synergy synchronizes the patient's molecular profile with targeted interventions, enabling real-time and precise evaluation of therapeutic efficacy.

Clinical Implications and Potential for Implementation

An approach focused on early diagnosis and personalized therapy for PCOS has important clinical implications, given that PCOS is a heterogeneous endocrine-metabolic disorder with a broad spectrum of symptoms and a risk of long-term complications such as insulin resistance, type 2 diabetes, and cardiometabolic disease.[27,28] Earlier diagnosis allows for more accurate stratification of PCOS phenotypes and the selection of therapies tailored to an individual's metabolic and hormonal profile and reproductive goals, thereby potentially improving treatment effectiveness and patient adherence. This approach aligns with the latest international consensus and guidelines emphasizing the importance of individualizing PCOS care as part of evidence-based long-term management.[29]

In developing countries, including Indonesia, the implementation of this strategy holds great potential given the significant prevalence of PCOS among women of reproductive age; however, it still faces constraints related to cost, variations in clinical regulations, and the readiness of technology and healthcare infrastructure, particularly at the primary care level. Additionally, a lack of training for healthcare workers, limited access to diagnostic tests, and suboptimal integration of clinical data further slow the adoption of early diagnosis and personalized therapy approaches.[30] Therefore, successful implementation in Indonesia requires strengthening health policies, enhancing human resource capacity, and adapting cost-effective technologies to align with the context of the national health system.

Limitations and Directions for Future Research

TPP+ targeted quercitol nanoliposomes represent a theoretical innovation that should be prioritized for comprehensive in vitro and in vivo validation to characterize their safety profile, biodistribution, and efficacy in targeting mitochondria. The primary focus of research should be on elucidating the molecular mechanism of action regarding how this nanoliposomal system is able to suppress oxidative stress and effectively reduce the release of cf-mtDNA in experimental PCOS models before proceeding to clinical trials in humans. Furthermore, dose-finding studies and pharmacokinetic evaluations are required to ensure that fluctuations in cf-mtDNA levels detected via liquid biopsy can serve as a valid and accurate real-time indicator of therapeutic response. Ultimately, longitudinal research will be crucial to transforming this theranostic concept from the bench-side stage into clinical applications capable of providing personalized solutions for the measurable improvement of metabolic function and restoration of fertility in PCOS patients.

CONCLUSIONS

PCOS is a complex endocrine-metabolic disorder closely associated with mitochondrial dysfunction and oxidative stress as the primary triggers of its clinical manifestations. The use of cell-free mitochondrial DNA (cf-mtDNA) via a liquid biopsy approach offers a more objective,non-invasive,and age-agnostic diagnostic solution compared to conventional diagnostic criteria. From a therapeutic perspective, the combination of quercetin and inositol in a TPP+ targeted nanoliposome delivery system demonstrates strong synergistic potential to precisely improve metabolic and hormonal profiles directly at the organelle level. This theranostic integration enables real-time monitoring of treatment efficacy through fluctuations in cf-mtDNA levels, thereby creating a more personalized, measurable, and effective PCOS management strategy for patients.

RECOMMENDATIONS

Implementing this strategy requires multidisciplinary collaboration. Researchers and academics are encouraged to conduct further in vitro and in vivo studies to validate the safety and efficacy of mitochondrial targeting, as well as the accuracy of cf-mtDNA as a biomarker of therapeutic response, through longitudinal clinical trials. For clinicians, this approach can serve as the basis for developing more objective and personalized early diagnosis protocols, particularly for adolescent girls. Meanwhile, the government and policymakers in Indonesia need to strengthen regulations and develop cost-effective technologies so that healthcare services, including primary care, are ready to adopt these diagnostic and therapeutic innovations.

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