



Urinary volatile metabolomics as a viable alternative diagnostic tool for polycystic ovary syndrome: An exploratory hypothesis



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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a metabolic disorder prevalent globally. Female infertility cases are also on the increase during the recent times which almost matches with the increasing incidence of PCOS. The NIH-USA-defined symptoms for clinical confirmation of PCOS include oligo-ovulation, elevated androgen level and presence of cysts in the ovary. Therapeutic approaches to PCOS require confirmatory diagnostics such as measurement of hormones and ultrasound scan of the ovary, which are in part, invasive. Conversely, the volatile organic compounds (VOCs) that are present in body fluids (urine, feces, saliva, etc.) and exhaled breath are reported to be endogenously altered in diseased state, which may be indicative of diseases including cancer. We hypothesize that the hindered metabolic state in PCOS condition would conditionally alter the VOCs that eventually are excreted in urine, which may offer a template to develop a viable and non-invasive diagnostic tool.

Introduction

Polycystic Ovary Syndrome, also called as “Stein-Levanthal syndrome”, is the most prevalent endocrine disorder in 5–10% of women in the reproductive age [27,4]. The primary characteristic feature of PCOS is clustering of underdeveloped follicles (referred to as cysts) in the ovaries. The immature follicles, filled with follicular fluid, are smaller in size but higher in number that eventually make the ovary larger than normal. This is accompanied by hypersecretion of androgens from theca cells and hyposecretion of estrogen from granulosa cells of the ovary [36]. The final revised criteria recommended for diagnosis of PCOS include hyperandrogenism (hirsutism/hyperandrogenemia), ovarian dysfunction [oligo-/an-ovulation/polycystic ovaries (PCO)] and exclusion of other androgen excess or related disorders [5].

Causes and clinical signs of PCOS

PCOS is a multi-factorial disorder, which may be influenced by environmental factors, genetic predisposition, hyperinsulinemia [14], strong stimulation of adrenals in childhood, contraceptive pills, medications that increase prolactin secretion, hormonal imbalance, neuroendocrine abnormalities, chronic inflammation, autoimmune disorders

of ovary, pancreas, thyroid and adrenal glands, and stress [15]. Yet, the primary cause for PCOS is unknown. The common clinical symptoms of PCOS include menstrual irregularities, hirsutism, acne, temporal baldness, changes in ovarian synthesis of estrogen-testosterone ratio and other hormones, hypertension, diabetes, dyslipidemia, hyper-pigmentation on neck, armpits, vulva and/or under the breast, etc. [17].

Diagnosis of PCOS

The analysis of serum androgen, assessment of menstruation/ovulation frequency and examination of ovary by ultrasound scan are crucial diagnostics in PCOS. The pelvic or trans-vaginal ultrasonography clinically confirms the PCOS condition by localizing at least one ovary with 12 or more follicles, each 2–9 mm in diameter, and absence of a dominant follicle. The quantification of androgens together with fasting lipid profile and glucose tolerance test [Oral Glucose Tolerance Test (OGTT) and Intra-peritoneal Glucose Tolerance Test (IGTT)] are also used as complementary diagnostics in PCOS [29]. Even though the available diagnostic methods aid in prompt and precise diagnosis of PCOS, the methods are often invasive and expensive. Albeit scientific and clinical research prompted the development of novel diagnostics for PCOS, a reliable, inexpensive, and non-invasive tool has

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not yet been developed. The early diagnosis of PCOS helps in effective patient management.

Volatile metabolomics

Metabolomics, in general, is the study of metabolites or chemical fingerprints that occur during metabolism. Metabolic profiling provides an overview of the cellular and physiological status' about an individual in brief and, therefore, widely recommended in clinical settings to diagnose health status. A plethora of studies documented volatile metabolomics as one of the key branches of metabolomics [26]. Recently, the advancements in methodological approaches (extraction and analytical methods), for instance Tenax and Solid-phase Microextraction (SPME), are considered important in volatile metabolomics. These methods are highly promising and require no solvent, and can be applied to variety of biological matrices for the extraction of volatile compounds. In parallel, enhanced features and capabilities of mass spectrometers, and improved statistical methods (e.g. Principal Component Analysis) have rendered the volatile metabolomics as an indispensable tool in comprehending the physiology.

Volatile metabolomics in body secretions – a promising tool in disease diagnosis

The use of smell is in practice by diagnosticians from the very BC, as Hippocrates used the foul smell associated with the feces to diagnose tuberculosis in patients. Later, it was revealed that odoriferous substances emanating from body secretions and breath primarily contain volatile compounds that reflect physiological conditions of animals and humans. In diseased condition, the volatile compounds are considerably altered; therefore, the study of volatile metabolome in body secretions of diseased individuals is used to develop 'candidate biomarkers' [35,13]. Putting forward the hypothesis that endogenous volatiles change during disease, Garner et al. [18] identified the candidate VOCs present in the feces of patients with gastrointestinal disease. The fecal volatile compounds indicative of inflammatory bowel disease were also identified (Crohn's disease and ulcerative colitis) [3]. A pilot study was performed to detect sepsis-related volatiles in feces of infants and proven to be effective [7]. In this direction, recently, numerous studies have been conducted on biological secretions and volatile biomarkers that would indicate deadliest diseases, injuries, infections and various cancers [26].

Urine- a potential source of volatile metabolomics that reveal disease

Urine in biomarker development studies has a number of merits such as collection is non-invasive, large volume of secretion, minimal contamination by exogenous/environmental volatiles, and storability for extended periods of time. Human urine has been reported to contain at least 3079 detectable metabolites that include 25 super classes of compounds, in that fatty acid and lipid contribute significant number of chemicals [10]. Urine contains potential substances that could be used as biomarkers of health conditions and is considered as "window into individual health". Among various elements in the urine, VOCs are of major interest for scientists and diagnosticians. From this sense, VOCs are identified and many diseases are correlated with candidate VOCs. The VOCs were identified in the urine of lung cancer patients, and a volatile-based colorimetric assay differentiates the urine from infected and normal individuals [31]. In tuberculosis-infected individuals, specific urinary volatile compounds were identified, and a VOC-based colorimetric assay was developed [30]. Batty et al. [6] identified the volatile biomarkers in the urine of colorectal cancer patients. The urinary volatile metabolome of renal cell carcinoma patients revealed consistently varied volatile compounds as biomarkers [33]. The urinary volatile profile varies greatly between overweight/obese and healthy

control children [12]. The urine of B-cell non-Hodgkin's Lymphoma patients were analysed for volatile compounds and candidate VOCs were identified [25]. However, no study has been performed hitherto to analyze or identify the volatile metabolome of urine in PCOS women.

Estrogen and androgen uniquely regulate urinary volatile compounds

Estrogen-dependent volatile compounds were identified in the urine of female mice [38]. In pine voles, urinary volatiles of estrogen-administered and ovariectomised mice were different [9]. It has also been evidenced that estrogens alter the urinary volatiles in wolf [37]. Similarly, testosterone is known to have influence over the urinary volatile compounds. Physiologically, intact male mice contain specific volatile compounds in urine that were absent in immature or castrated mice attributing the role of androgen in some urinary volatile compounds [19,1]. Urinary volatile compounds dependent on testosterone were also identified in cats [32]. Based on these evidences, we propose androgen and estrogen are crucial in contributing to the volatile compounds excreted through urine. Further, the lower urinary tract of women contains receptors for estrogen and progesterone [8], which may unequivocally contribute to alteration of volatile compounds that are excreted in the urine. Moreover, steroid hormone receptors were also found in kidney tubules [16], which could also have participatory role in changes of urinary volatiles.

Merits of volatile compounds in disease diagnosis

The VOCs in living organisms are a wide range of stable chemicals of endogenous origin and blood-borne. These compounds and their subtle alterations during disease are apparently reflected in body fluids and exhaled breath. Indeed, the perturbed biochemical events or the infectious agent or both together alter the VOCs and, thus, each disease owes to specific pattern/signature of compounds. Moreover, these endogenous VOCs are prone to minimal contamination with other external and environmental factors. The use of volatile metabolomics in diagnostics is of interest in recent times, and offers rapid and potentially inexpensive approaches than diagnostics of current scenario. Also, the low-molecular weight of VOCs enables the development of sensitive and viable point-of-care devices. The sampling technique of body fluids is painless, non-invasive, and reproducible. Moreover, detection of specific VOCs does not involve complicated assays or instruments that require extensive sample preparation methods.

Volatile-based sensors

Although the volatile-based sensor development is in infancy, the detection limits (sensitivity and specificity) are promising. The sensor to be developed should be sensitive in capturing the analyte even at its lowest concentration. Also, it should react rapidly and differently to minor changes in the samples to be analyzed. The sensor should revert to baseline when not in use and easily be reproducible [11]. Although many diseases have been studied, only few results have been translated into sensor devices. Also, the sensor devices are most often used as a complementary technique of diagnosis along with the existing diagnostic techniques.

Potential electronic sensors developed

The candidate compounds are used to develop point-of-care sensor devices (e.g. e-nose), in which the volatile compounds react and rapidly indicate the positivity. A breath-based sensor (hearts breath) currently in use for the diagnosis of heart transplant rejection. Apieron INSIGHT™ eNO System is used to diagnose asthma using breath volatiles [20] and a gas sensor is available to discriminate the urine samples that are infected with different microbes [21]. Osmotech Microbial Analyzer-

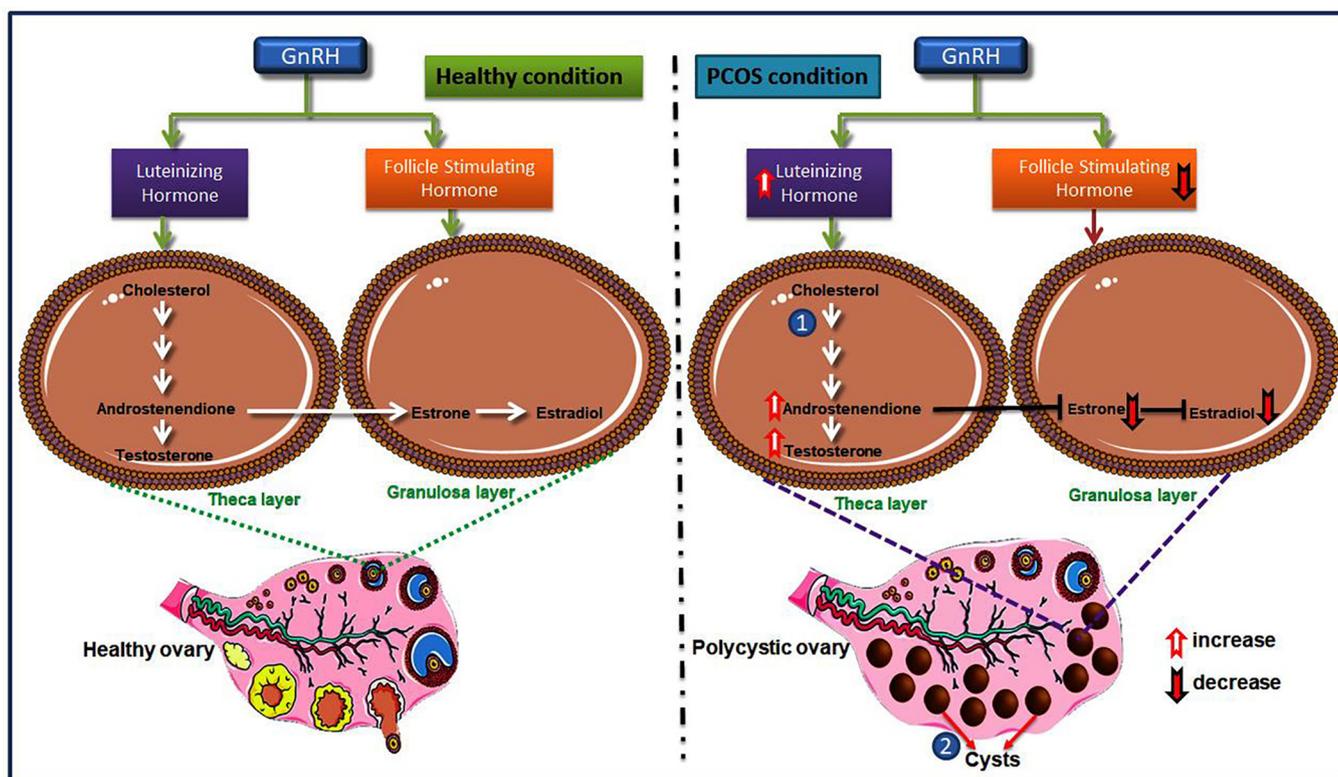


Fig. 1. Schema of plausible sequelae leading to altered volatile organic compounds in the ovary during PCOS condition. Volatile compounds may originate from the checkpoints (encircled numbers). (1) The hormonal imbalance (estrogen-androgen ratio) together with perturbed cholesterol metabolism, and (2) The follicular fluid in immature follicles (cysts) in the ovary.

Bacterial Vaginosis (OMA™-BV) for the detection of bacterial vaginosis using VOCs from vaginal swab is approved by US-FDA in 2003. A breath volatile-based diagnostic sensor was developed recently to detect and discriminate 17 different types of diseases including some cancers, neurodegenerative diseases, etc. [34].

Databases developed for volatile metabolite analysis

Especially, dedicated databases have been useful in comprehensive interpretation of volatile compounds; human metabolome [39], Wishart et al. [40,24], human urine metabolome database [10], (<http://www.urinemetabolome.ca/>), microbial volatile organic compounds (mVOC) [28,22], and volatile organic compounds in cancer (VOCC) [2,23].

Hypothesis

The volatile molecules are carried by blood to distant organs, and subsequently, in part, excreted via urine, skin emanations, exhaled breath, feces, saliva, mucus and other body/glandular secretions. Urinary volatiles represent metabolic changes and identified as candidate biomarkers. Nevertheless, it has not been adequately analyzed to identify biomarkers of PCOS. In normal ovary, LH receptors present in theca cells of pre-antral follicles respond to LH surge by producing androgens from cholesterol through progesterone. The androgens thus produced will be further converted into estrogen in the granulosa layer eventually regulates the secondary sexual characteristics, menstrual cycle, etc. In PCOS condition, the LH receptors in theca cells of the pre-antral follicles respond to LH surge, and produce enormous androgen but fail to convert it into estrogen in granulosa cells that together attribute to pronounced changes in estrogen-androgen ratio. Under this reduced estrogen level, the immature follicles (2–9 mm) fail to transform into mature follicles (16 mm or more). Ultimately, the internal

fluid components of the follicle start to aggregate, and are formed into cysts. From these perspectives, we speculate two possible means by which volatile changes could occur:

- i) the hormonal imbalance (estrogen-androgen ratio) together with perturbed cholesterol metabolism; and
- ii) the follicular fluid in immature follicles (cysts) in the ovary (Fig. 1).

The volatiles thus produced/altered should be transported endogenously by blood to the excretory systems, and certainly be excreted in body fluids (urine, feces, saliva, breath, body odor, vaginal secretions, etc.). In this way, in support of existing literatures, we speculate that urine as the most potential source of PCOS-indicative volatiles. Altogether, we propose utilizing urine as a potential target to identify candidate VOCs and for the development of feasible non-invasive diagnostic of PCOS.

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Conflict of interest

The authors declare no conflict of interest.

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