Review Article

Cancer metabolomics: A tool of clinical utility for early diagnosis of gynaecological cancers

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Gynaecological cancers are the major cause of cancer-related deaths in Indian women. The poor prognosis and lack of symptoms in the early stages make early cancer diagnosis difficult. The absence of mandatory screening programmes and the lack of awareness pose to be a real challenge in a developing economy as India. Prompt intervention is required to enhance cancer patient survival statistics and to lessen the social and financial burden. Conventional screening and cytological techniques employed currently have helped to reduce the incidence of cancers considerably. However, these tests offer low sensitivity and specificity and are not widely used for risk assessment, leading to inadequate early-stage cancer diagnosis. The accomplishment of Human Genome Project (HGP) has opened doors to exciting 'omics' platforms. Promising research in genomics and proteomics has revolutionized cancer detection and screening methodologies by providing more insights in the gene expression, protein function and how specific mutation in specific genes corresponds to a particular phenotype. However, these are incompetent to translate the information into clinical applicability. Various factors such as low sensitivity, diurnal variation in protein, poor reproducibility and analytical variables are prime hurdles. Thus the focus has been shifted to metabolomics, which is a much younger platform compared to genomics and proteomics. Metabolomics focuses on endpoint metabolites, which are final products sustained in the response to genetic or environmental changes by a living system. As a result, the metabolome indicates the cell's functional condition, which is directly linked to its phenotype. Metabolic profiling aims to study the changes occurred in metabolic pathways. This metabolite profile is capable of differentiating the healthy individuals from those having cancer. The pathways that a cell takes in turning malignant are exceedingly different, owing to the fact that transformation of healthy cells to abnormal cells is linked with significant metabolic abnormalities. This review is aimed to discuss metabolomics and its potential role in early diagnosis of gynaecological cancers, *viz*. breast, ovarian and cervical cancer.

Key words Biomarker - cancer metabolomics - early cancer detection - metabolic profiling - NMR

Cancer is a condition of DNA dysregulation that is influenced by both internal and external causes. The exogenous factors responsible for carcinogenesis are infection, tobacco addiction, unhealthy diet and physical inactivity and endogenous factors include inherited genetic mutation, hormones and immune conditions[1]. A survey conducted by GLOBOCAN in 2018 indicated 18.1 million new cancer cases

and 9.6 million cancer-related deaths globally, compared with 14.1 and 8.2 million, respectively, in 2012[2]. In 2018, GLOBOCAN reported nearly 1.16 million newly diagnosed cases and 0.78 million deaths due to cancer in India[2]. Breast cancer is now the leading cause of mortality in Indian women, followed by cervical and ovarian cancer[3,4]. These cancers can be prevented if diagnosed early[5]. Poor prognosis and lack of symptoms coupled with lack of awareness and screening programmes are posing as real challenges in a developing country like India for early diagnosis of cancer. A timely intervention is needed not only to improve survival rates for cancer patients but also to reduce social and financial burden[6].

Various screening and cytological techniques are employed, *viz.* Pap smear test for cervical cancer, cancer antigen (CA-125) for breast and ovarian cancer. Radiology based screening like sonography and mammography is recommended for females with early clinical symptoms or those having strong family history of cancers. These screening techniques have helped in reducing the incidence of cancers considerably. Sankaranarayanan *et al*[7] showed that performing 4 tier cancer awareness and screening by employing low impact visual inspection using acetic acid, cytology and human papilloma virus (HPV) testing reduced cervical cancer mortality by 31 per cent in the screening group. However, these tests offer low sensitivity and specificity and are not widely used for risk assessments. The deciphers of the human genome have facilitated application of various 'omics' platforms for early cancer diagnosis. Thus, in this review we discuss metabolomics and its potential role in early diagnosis of cancer of breast, ovarian and cervical cancers.

Limitation of conventional techniques

Conventional techniques used for screening gynaecological cancers have led to considerable reduction in the numbers of cancer incidence. However, these techniques have significant drawbacks. For cervical cancer screening, Papanicolaou (Pap) smear is widely used and its accuracy is based on the preparation of a good smear and correct interpretation by an expert. It cannot efficiently detect squamous cell abnormalities and has a high probability of incorrect negatives due to interference from blood cells on glass slides[8]. Similarly, CA-125, although widely used for screening ovarian tumours, generally gives false-negative results during the early stages. This may be because the expression of the antigen occurs in later stages[9]. In such cases, if the

patients fail to follow up in spite of having recurrent clinical symptoms, the diagnosis is missed. Therefore, histopathology is still considered as the gold standard for screening and diagnosing. Histopathology is highly accurate in diagnosing cancer, but again, it is not used for risk assessment. The limitation of histopathology is that it is invasive, inconvenient, time-consuming and costly. It cannot be applied for mass screening. Further, the results depend on the sampling of tissue biopsy and require experts for interpretation. The traditional radiological methods such as X-ray, computed tomography (CT), magnetic resonance imaging (MRI) are generally used in conjunction with histopathology to detect and locate advance stages of cancer. These techniques, however, cannot capture lesions in early stages or cancers with no imaging abnormalities[10,11]. As a result, a general cancer screening test that is cost-effective, minimal invasive, highly sensitive, specific and capable of being adopted in India's rural population is required[12]. The current diagnostic methods used for early-stage cancer diagnosis lack these qualities and are inadequate.

Exploring 'omics' platform for cancer detection

The advancement in 'omics' has opened the doors to molecular screening and diagnosis based on individual's genetic make up. The whole-genome sequencing has helped identify specific sets of genes with respect to their function, thereby facilitating to target genes/specific panels responsible for this phenotype and broadly to study variants associated with it[13]. It has revolutionized the approach used to screening and diagnosis of cancers by providing exciting 'omics' platforms at a molecular level.

These platforms are widely used to carry out analysis of genes (genomics), proteins (proteomics), mRNA (transcriptomics) and metabolites (metabolomics) in a given biological sample[14]. In the previous two decades, significant work has been conducted in the field of genomics and proteomics, leading to many ground-breaking discoveries. More insights about the cell functionality at a molecular level are achieved[15]. However, still, there is hindrance to translate this information, in clinical applicability because of a number of factors. The prime hindrance is low sensitivity, diurnal variation in protein, poor reproducibility, analytical variables and accurate interpretation of data[16]. The data are still not incorporated into definite clinical context[17]. The comprehensive information on cellular networking

in terms of protein variability due to splicing and post-modification is lacking in both genomics and proteomics[18]. Further, both of these areas are expensive and labour intense[19]. This has eventually shifted the focus to metabolomics, which is a much younger platform compared to genomics and proteomics. In addition, the data generated in each omics differ vastly. Genomics involves a coverage of approximately 30,000 genes and 30,000 transcriptomes, proteomics involves >100,000 while metabolomics involves ~6500 metabolite analysis[20]. The complexity involved in metabolomic analysis is much lesser as the size of data generated decreases.

Metabolomics is a detailed assessment of every metabolite which exists in the given specimen[21]. It comprises simultaneous identification and quantification using innovatory analytical technologies coupled with statistical and multivariate methods[22] to race the changes in biological samples[23-25].

The metabolome is the comprehensive set of all low molecular weight metabolites generated by cells during metabolism that serves as a readout of cellular activity and physiological status[26]. Thus, the metabolome represents the downstream products of the cell metabolism which more accurately corresponds to its phenotype[19]. Metabolic profiling or metabolome analysis is emerging as a potential tool for better understanding of metabolic systems and how they respond to various stressors such as disease state[10].

Historic association of metabolism and cancer

More than 90 years ago, the connection between metabolism and cancer was established. This became well known as the 'Warburg effect', where increased uptake of glucose and fermentation of glucose into lactate in the presence of oxygen were observed. This metabolic distortion has since then been considered as the hallmark of cancer cells[27]. Sir Otto Warburg received the Nobel prize for this discovery in 1931. Later on, after many years, this signature metabolic change was explored as a marker for cancer diagnosis. The widely used cancer positron emission tomography (PET) scan imaging system was designed based on the principle of Warburg effects. Extensive research later in this field indicated that cancer metabolism is more complex than it was depicted earlier by Warburg[27]. Studies have shown that tumour cells undergo aerobic glycolysis along with Warburg effect. This is achieved either by activation of the oncogene (*AKT, MYC* and *RAS*) or loss of function in tumour-

suppressor genes (succinate dehydrogenase and fumarase hydratase). This is exacerbated by a new mechanism that stabilizes hypoxia-induced factor (HIF) either as an adaptive response to hypoxic environment or through pathways that stabilize HIF in non-hypoxic situations[28]. Such perturbances in pathways contribute to oncogenic phenotype.

Cancer metabolomics

Cancer metabolomics is a branch which involves comprehensive analysis of metabolites in cancer. The basic principle is to study changes occurring in metabolic pathways, premised on the theory that the transition of healthy cells to abnormal cells is linked to significant metabolic perturbations. A dysplastic cell's path to turning malignant is exceedingly diverse. As a result, a cell's metabolite profile is more linked to reflect the cell's current state and can be linked to its phenotype. This metabolite profile is capable of differentiating the healthy individual from those having cancer[19].

There is a two-pronged approach while applying metabolomics to cancer diagnosis. One of it is biomarker discovery or untargeted metabolomics, in which global detection and quantification of all metabolites present in the sample are carried out. Further, these metabolites are mapped to metabolic networks and pathway to know any significant perturbation with reference to controls. The probability of discovering novel biomarkers without previous knowledge is possible using this approach[29]. The other approach is targeted metabolomics, in which known metabolites that lead to disease stage are traced and quantified. The chances of false-positive result are reduced as the structure, molecular formula and biochemical nature of the metabolites are known[30]. The overall precision is achieved in targeted metabolomics as compared to non-targeted metabolomics. To sum up, both approaches are interdependent and lead to high throughput and large coverage of metabolic markers[31].

It is very important to know the composition and nature of the key biomarkers in the metabolomic analysis, as the analytical platform should be selected based on it. The selection of an appropriate analytical platform is important in metabolomics.

Role of different analytical platforms in metabolomics

A metabolite is a diverse group comprising lipids, amino acids, peptides, nucleic acids, organic acids,

vitamins, thiols and carbohydrates generally weighing less than 1200 Dalton, which makes the global analysis a task. A single platform is thus insufficient, considering the diverse nature of the metabolites[32]. Combined or integrated platforms are used to optimize the outcome of analysis by allowing sensitive and accurate identification of hundreds of metabolites present in samples[32]. Multiple analytical platforms are available including gas chromatography (GC), high performance liquid chromatography (HPLC), ultra performance LC, capillary electrophoresis coupled to mass spectrometry (MS) and nuclear magnetic resonance (NMR). The most critical step is choosing an appropriate analytical platform, which is determined by the biological material to be examined and the expected outcomes. Different platforms vary in their principle, sensitivity, specificity, reproducibility and costs[19].

The two major platforms used for metabolomics application are NMR and MS. The phenomenon of NMR occurs when nuclei in a magnetic field absorb and re-emit electromagnetic energy. This energy has a specific resonance frequency that is determined by the magnetic field strength and the magnetic characteristics of the atoms' isotopes. A predictable spectrum of radiation is released, resulting in a distinct pattern of peaks that is unique to each molecule. NMR offers high reproducibility of 98 per cent which is higher than any other technology and allows detection in both liquid forms and in intact tissues[33]. Minimal sample preparation and quantification of compounds in mixtures or unknown metabolites is possible, unlike MS. The compounds having difficulty to ionize or with identical masses including those with different isotomoper distribution can be easily analyzed by NMR[33]. NMR is high throughput and robust, allowing 200-300 samples analysis per day[19]. Conversely, it is less sensitive than MS and low abundance metabolites are not detected.

MS is a technique for identifying metabolites that involves the production and separation of ions based on their mass-to-charge ratio[34]. MS is highly sensitive and shows good resolution permitting simultaneous detection of thousands compound[33]. MS is frequently used in conjunction with separation techniques. It does not differentiate isobaric metabolites on its own, hence GC-MS and LC-MS are used. GC-MS is beneficial for non-targeted analysis for volatile compounds or compound that can be volatile after chemical derivatization[33], while LC-MS is used for identification of liable, non-volatile and non-polar compounds. During sample derivatization, certain important metabolites which are highly unstable in nature can be lost, resulting into alteration in sample composition before these are quantified. Thus, proper handling and sound knowledge about the target compound are important. Second, the internal standards used are specific for targeted compounds and thus make it impractical for metabolite biomarker discovery. Another major hurdle is quantification and identification of key biomarkers contributing to a disease state[35].

Each platform has its own strengths and weakness. We need to synergistically use their strength to optimize the biomarker detection for early intervention and diagnosis of cancer. This can be achieved by using metabolomics approach, considering the vast number of platforms available. Till date, various platforms have been explored based on cancer and sample type as shown in Table[36-53].

Potential of metabolites as biomarker in cancer diagnosis

Many biomolecules, products and intermediates of pathways are found to be deranged in cancer. Breast cancer patients have been found to have elevated choline alterations in oestrogen metabolism, as well as a significant increase in metabolites associated with oxidative DNA impairment and the methylation process[54,55]. Fong et al[43] found decreased level of tricarboxylic acid cycle metabolites and amino acids and increased level of carnitine and products of fatty acid metabolism in addition to metabolites responsible for oxidative DNA damage. Elia et al[46] reported 3-hexanone, hexanal, dodecane, 4-methyl and 3-ethylcyclopentanone in patients with cervical intraepithelial neoplasia (CIN-I). Another study[46] found eight metabolites [Cer (d18:1/16:0), PC (15:0/16:0), PC (16:0/16:0), PE (16:0/20:0), PC (14:0/20:0), PS [17:0/22:2(13Z,16Z)], PG [21:0/22:4(7Z,10Z,13Z,16Z)] and SM (d18:1/20:0)] as potential biomarkers for cervical cancer diagnosis. Paraskevaidi et al[52] employed metabolomics to detect presence of high risk HPV strain and found to be 94 per cent sensitive and 83 per cent specific. Pappa et al[53] conducted studies on four cell line, one normal cell line and three cancerous cell lines positive for HPV 16 (SiHa HPV 16+), HPV 18 (HeLa HPV 18+) and one negative for HPV (C33A). Cell line positive for HPV 16 and 18 exhibited hallmarked Warburg metabolism and

Study	Cancer type	Biomarkers	Platform
Table. Overview of significant research carried out using metabolomics approach in breast, ovarian and cervical cancer			
Odunsi et al[36], 2005	Ovarian	Detection of EOC: 100 per cent Benign ovarian disease: 100 per cent Control from patients: 97 per cent Sensitivity: 100 per cent Specificity: 100 per cent	1H-NMR
Denkert et al[37], 2006	Ovarian	Enzymes regulating pyrimidine metabolism	GC-TOF
Woo et al[38], 2009	Breast, Cervical, Ovarian	BrCa: ↑ 5-hydroxymethyl-2-deoxyuridine and 8-hydroxy-2-deoxyguanosine, 5-hydroxymethyl-2-deoxyuridine, 8-hydroxy-2-deoxyguanosine Ov Ca: Non-specific↑in 1-methyladenosine, 3-methyluridine and 4-androstene-3,17-dione Cx Ca: Patterns were distinguished	GC/MS, LC-MS
Kim et al[39], 2010	Breast	Urine metabolites (multivariate classification)	GC/MS
Slupsky et al[40], 2010	Breast and Ovarian	Supressed TCA and urea cycle; ↓glucose; ↑amino acids transports	NMR
Oakman et al[41], 2011	Breast	Differential metabolites were obtained for metastatic, early patients and control	NMR
Li et al[42], 2011	Breast	Predicted presence of cancer with 69 per cent sensitivity and 94 per cent specificity	HR-MAS MR
Fong et al[43] 2011	Ovarian	Changes in glycolysis; β-oxidation of fatty acids, phenylalanine catabolism; aminobutyrate; isoforms of tocopherols; N-acetyl aspartate; N-acetyl-aspartly-glutamate	GC/MS, LC-MS/MS
Budczies et al[44], 2012	Breast	Detection rate for this biomarker: cytidine-5-phosphate/pentadecanoic acid ratio Sensitivity: 94.8 per cent Specificity: 93.9 per cent	GC-TOF-MS
Hasim et al[45], 2013	Cervical	Plasma-free amino acids	HPLC
Elia et al[46], 2015	Cervical	3-hexanone, hexanal, dodecane, 4-methyl and 3-ethylcyclopentanone	GC/MS
Huang et al[47], 2016	Breast	Found taurine, hypotaurine, glutamate and aspartate pathway metabolites	LC-MS/TOF, GC-TOF-MS
Ke C et al[48], 2016	Ovarian	EOC primary: 37 metabolites identified-abnormal lipid metabolism, energy disorders Post-surgical: 30 metabolites identified-oxidative stress markers Recurrence cases: 26 metabolites identified-↑amino acids and lipid metabolism	LC/MS
Yang W et al[50], 2018	Ovarian	2-piperidinone and 1-heptadecanoylglycerophosphoethanolamine Sensitivity- 73 and 97 per cent, respectively Specificity-83 and 60 per cent, respectively	UPLC/Q-TOF MS
Zhou et al[49], 2019	Cervical	Cer (d18:1/16:0), PC (15:0/16:0), PC (16:0/16:0), PE (16:0/20:0), PC (14:0/20:0), PS [17:0/22:2 (13Z,16Z)], PG [21:0/22:4 (7Z,10Z,13Z,16Z)] and SM (d18:1/20:0)	UPLC/Q-TOF MS

Contd...

Study	Cancer type	Biomarkers	Platform
His M et al[51], 2019	Breast	Acetylcarnitine (Positive association) Arginine, Asparagine and PC (inverse association)	QTRAP5500-MS
Paraskevaidi M et al[52], 2020	Cervical	Detection of high risk HPV strain Sensitivity-94 per cent Specificity-83 per cent	LA-REIMS
Pappa KI et al[53], 2021	Cervical	Perturbance in normal pathway was noted in cancerous cell line. SiHa and HeLa cell line took purine salvage pathway while C33A showed synthesis of cytidine through novel mechanism.	UPLC-MS/MS

EOC, epithelial ovarian cancer; NMR, nuclear magnetic resonance; GC, gas chromatography; TOF, time-of-flight; MS, mass spectrometry; LCMS, liquid chromatography mass spectrometry; HR-MAS, high-resolution magic angle spinning; MR, magnetic resonance; HPLC, high-performance liquid chromatography; TCA, tricarboxylic acid

was accordant with role of HPV protein E6. Further, SiHa and HeLa cell line took purine salvage pathway to sustain angiogenesis and C33A cell line underwent novel mechanism by synthesizing cytidine.

These underlying changes in the pathway and their reflection in phenotype, leading to disease stage such as cancer, are tracked and tapped in cancer metabolomics. These changes act as a biomarker indicating a perturbation in the normal pathway and its progression towards oncogenesis.

A few studies have narrowed down the biomarkers specific to particular cancer. Huang et al[47] found taurine, hypotaurine, glutamate and aspartate pathway as some critical biomarkers for early diagnosis of breast cancer using LC-MS/TOF (Time of Flight) and GC-TOF-MS. Acetylcarnitine was shown to be a potential biomarker for breast cancer. It exhibited positive correlation while arginine, asparagine and phoshotidylcholine levels were inversely proportional using QTRAP-MS[51] Urinary metabolic profiling was done on patients having breast and ovarian cancer and a unique metabolic profile was found[40]. A significantly different metabolite was generated in both the cancers which could be specifically correlated with clinical aetiology[40]. Woo et al[38] statistically validated that there was a significant difference in urinary metabolites concentration and there were separable metabolite signatures in breast, ovarian and cervical cancers.

Thus, it is possible to differentiate between gynaecological cancers using urinary metabolic profiling. Using a ultra-high performance liquid chromatography-quadrupole time-of-flight (UPLC/QTOF) MS-based metabolomics method and multivariate data analysis, Yang and colleagues[52] discovered 12 biomarkers that might be related with aberrant fatty acid oxidation and phospholipid and bile acid metabolism in ovarian cancer. A multivariate logistic regression model was used to validate two of the discovered metabolites (2-piperidinone and 1-heptadecanoylglycerophosphoethanolamine). These findings contribute to the understanding of the pathophysiology of ovarian cancer and may help in clinical diagnosis and treatment[5]. Denkert et al[37] gave more insights as they could differentiate borderline ovarian tumours and ovarian carcinoma based on metabolite profiles of samples, using three different analytical platforms GC/MS, LC/MS and LC-MS/MS. Fong et al[43] obtained the metabolome of the healthy ovary and metabolome during transition to cancer, i.e. primary and metastatic stage.

Ki et al[48] employed MS for methodological investigations in patients with advanced-stage ovarian cancer, patients with post-surgery and those showing recurrence of ovarian cancer. These three groups revealed a considerable difference in their metabolite concentration. Specific 37 metabolites were found to be higher in patients with epithelial ovarian carcinoma compared to controls, while eight returned to normal following surgery and four were found to be elevated again in patients with relapse. Such signature changes hold great potential in the diagnosis of cancer along with its stages and relapse and can in turn be used to monitor the efficacy of surgery making personalized care and management possible.

The metabolomic platform's translational utility was investigated in clinical settings as shown in the Table. Sensitivity and specificity for cytidine-5-phosphate/pentadecanoic acid ratio as a biomarker in breast cancer was accessed. It was the most significant key biomarker. This biomarker was

detected with a sensitivity of 94.8 per cent and 93.9 per cent specificity[44].

Oakman *et al*[41] grouped 44 patients with early - stage breast cancer who were in the pre- and post-operative stages. Their serum profiling revealed pre-operative patients with 72 per cent predictive accuracy, 75 per cent sensitivity and 69 per cent specificity. Li *et al*[42], using orthogonal partial least squares discriminant analysis (OPLS-DA) multivariant model, proved that cancer and non-cancerous samples were distinguished with 69 per cent sensitivity and 94 per cent specificity in breast cancer detection. Odunsi *et al*[36] carried out serum analysis using NMR in ovarian cancer patients and a few healthy volunteers. The biomarkers found, showed 100 per cent sensitivity and specificity. Similarly, CIN and cervical squamous cell carcinoma were characterized using NMR and showed 91.6 per cent and 100 per cent sensitivity, respectively, using OPLS-DSA tool[45].

Further, advancement in computational biology and bioinformatics has facilitated integration of metabolomics with fluxomics for biomarker discovery. Using metabolomics has led to the discovery of a potential biomarker in a specific disease state after comparing it to a control group and fluxomics has helped to determine the rate of metabolite (*i.e.* molar per unit time) or metabolite turnover in a particular pathway[52]. Thus, synergically, both metabolomics and fluxomics approaches give the detailed picture of the potential biomarkers along with its reflux. The significance of a particular marker highlighting the perturbation in pathway is increased by two folds.

Merits and pitfalls of metabolomics

Metabolic profiling is fast becoming a new avenue to study biochemical refluxes arising due to responses to various host conditions and environmental stimuli. In cancer metabolomics, this biochemical reflux plays an important role in promoting and sustaining oncogenic state. Additionally, it has more advantages than conventional method. Metabolomic analysis can be carried out by obtaining biological samples such as blood, urine and biopsy tissue, which are procured by non-invasive or minimally invasive methods[10]. Further, a very small quantity of sample is required for the analysis, which can also be stored at –40 or –80°C for an extended period without alteration on subsequent analysis[56]. Early detection is possible even before clinical manifestation. The high sensitivity and the ability to predict cancer are added features of

metabolomics, which has been attributed to biomarker discovery[57].

Metabolomics holds an upper hand compared to other 'omics' sciences and has led to a significant advancement in metabolite identification, pattern recognition and statistical analysis. This has the ability to detect cancer sooner, when it is still curable[58]. Although the promising outcome is visible, yet there are persistent challenges in translating this information into clinical practice and cancer diagnosis. Metabolite analysis is the major hindrance right from standardization of protocol to data analysis[19]. Accurate interpretation from complex data is a difficult task[59]. In addition, confounding factors such as diet, age, gender, ethnicity, lifestyle, stress, drugs and environment may affect the metabolite profile. Barriers include regulatory constraints and a lack of finances to identify new biomarkers and further validate existing indicators, as well as markers that are difficult to quantify[10]. Certain software-based methods are developed in pre-processing steps to minimize the confounding effects, and orthogonal signal correction and variable stability scaling are some examples[60,61]. On the analytical side, constant efforts are made to refine sample preparation, processing, and experimental settings, as well as steps to manage data[10].

Conclusion

Metabolomics holds great potential to serve as a good platform for early cancer diagnosis. Metabolic profiling through the means of metabolite analysis has led to the discovery of clinically relevant biomarkers[61]. These biomarkers can further be categorized as prognostic biomarkers, diagnostic biomarkers and tumour biomarkers depending upon which clinical stage they manifest in. Some biomarkers are global markers as they are present in various types of cancer. Still, they are products of changed metabolism and hallmark of cancer cells. There are a few biomarkers which are uniquely linked to a particular cancer. These biomarkers are prime targets, and if we could validate the projected biomarkers, detection of all three gynaecological cancers can be possible in a single analysis. The main goal is to diagnose a particular cancer at an early stage so that it can be cured. Owing to constant research and development in this area, the identification of more such clinically relevant biomarkers has been possible. However, the hurdles such as limited literature, inadequate research, analytical technique limitation and technical difficulties still exist, which need to be overcome.

At present, apart from gynaecological cancers, renal cell carcinoma[62], human bladder cancer, gastric cancers and prostate cancer[63] have been explored using metabolomics approach. The outcome is promising as a unique metabolic profile is exhibited in a particular cancer. Substantial research in this field shall validate more biomarkers responsible for oncogenesis in specific cancers, and it may be soon possible to anticipate a single test to screen multiple cancers based on their metabolic profile.

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