DECODING THE ENDOGENOUS METABOLOME OF BREAST CANCER: ANALYTICAL APPROACHES AND CHEMOMETRIC INTERVENTION. A SYSTEMATIC REVIEW

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Abstract
Breast cancer (BC) is the leading cause of cancer mortality in females worldwide. Metabolomic approach has shown broad potential in recognizing the carcinogenic metabolites. This study aimed to systematically review cellular and clinical metabolomic studies in the past decade on BC. We summarized the pathways and metabolic biomarkers associated with BC. Scopus, PubMed, SAGE Journals, and Cochrane Library databases were searched for research papers on metabolomics of BC from January 2010 to January 2021. Two reviewers evaluated the data and study eligibility. The search identified 924 records. In total, 51 studies were included in the review (33 clinical and 19 cellular research) based on inclusion and exclusion criteria. Relevant data were extracted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines. The selected metabolomics studies analyzed tissue, serum, plasma, urine, and breath. A total of 21 metabolites (reported in ≥ 3 studies) were found to be prevalent in BC. Metabolite's alterations involving glutamate, glutamine, lactate, choline, and taurine provide evidence of tumorigenesis. Glutaminolysis presented as the most significant pathway which highlighted the correlation of glutamine, glutamate, and glutaminase enzyme as potential biomarker for BC diagnosis. In conclusion, metabolomics enables in-depth identification of BC metabolic profile. The relative non-invasion and advantages of convenience in comparison with tissue biopsy and imaging screening, considered metabolomics as a relevant tool in early BC diagnosis. Collectively, this may lay foundation for understanding the progression and development of BC.

Keywords: Breast cancer, metabolomic, metabolites.

INTRODUCTION

Breast cancer (BC) is changing the global landscape of cancer. Statistics released in December 2020 by the International Agency for Research on Cancer (IARC) reported that female BC has overtaken lung cancer as the most frequently diagnosed cancer worldwide, with an estimated 2.3 million newly diagnosed cases (1). It ranked first as the highest incidence cancer in 159 of 185 countries and fifth as the leading cause of cancer mortality with 685,000 deaths based on the data from GLOBOCAN 2020.

BC has primary molecular subtypes which can be categorized based upon their receptor status, including estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2). Based on gene expression profiling and immunohistochemistry analyses, these subtypes can be further categorized as luminal type A (ER-positive/PR-positive, HER2-negative), luminal type B (ER-positive/PR-negative, HER2-positive), HER2-enriched (ER-negative/PR-negative, HER2-positive) and basal-like (ER-negative/PR-negative, HER2-negative) (2) .

The pathophysiology of BC is multidimensional. It exhibited significant heterogeneity in disease and biological behaviour, in which different individuals could present with substantially different treatment-related toxicity and outcomes to treatment (3). Previous studies have evidenced that timely treatment and early diagnosis of BC could exert a significant outcome in improving the BC prognosis (4). Over the past decades, considerable progress has been made in the treatment and evaluation of patients with BC, leading to a nearly 40% reduction in mortality due to the improvement of treatment and prevention strategies (5). Additionally, a study by Hadi *et al.,* (2017) has reported that five-year survival rates can be improved to more than 90% by the early-stage diagnosis as compared to 15% in women diagnosed at an advanced stage of BC (6).

However, the current management approach in metastatic and advanced BC is still limited due to the lack of available prediction markers for early detection of treatment effects and outcomes (3,4). As the most widely used markers for BC, neither the ER, PR nor HER2 have satisfactory specificities and sensitivities for early diagnosis (4,5). Although annual screening of BC via digital mammography (DM) is considered an effective way to lower BC mortality in age-appropriate asymptomatic women, the sensitivity tends to rely on the tumor growth pattern and tissue density (7). Recent studies indicated that DM can potentially be replaced by the digital breast tomosynthesis (DBT) for early detection of BC, which demonstrated a prevalence sensitivity in the dense breast to some extent, however, this small-scale evidence concluded that it is still insufficient to confirm a shift from DM to DBT (6,8). Thus, novel convenient and effective techniques are urgently needed for early diagnosis of BC.

Within this context, the alternative strategy for BC intervention is metabolomics, a discipline that allows measurement of endogenous metabolic substances in response to external or internal changes in the body (4,9). This technique identifies a specific set of metabolites in biological samples under normal condition in comparison with altered conditions caused by environmental modulation, drug treatment, diseases, or dietary intervention (9). This gives rise to the discovery of valuable biomarkers for early diagnosis of various cancers and served as an effective technique for personalized medicine (4, 7).

MATERIALS AND METHODS

Identification

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as described by Liberati *et al*., 2009, comprise a four-phase flow diagram was applied in the study to screen and identify manuscripts related to metabolomic of BC (10). The diagram describes the identification, screening, eligibility, as well as the final included reports for the review (Figure 1). The first stage was the identification of journal articles. A systematic search was conducted on Scopus, PubMed, SAGE Journals, and Cochrane Library databases for relevant literatures published between 01/2010 up to 01/2021. The advanced retrieval used the following search terms: (“decoding” OR “deciphering” OR “interpreting” OR “translating” OR “figuring out” OR “sorting out” OR “unraveling” OR “understanding” OR “comprehending” OR “investigating”) AND (“metabolome” OR “metabolomic” OR “metabolite”) AND (“breast cancer”).

Screening

The second stage was employed by screening the identified records. The studies were all imported into Mendeley (Elsevier, version 1.19.4) for screening and duplicate removal. This platform run an automated duplicates removal by screening into the records. The remaining records were independently extracted into a standard Excel file for screening based on title and abstracts. The abstracts of the selected titles were then carefully studied to ensure the suitability of the articles.

Inclusion and exclusion criteria

Selection of articles was based on the inclusion and exclusion criteria. The criteria were determined by consensus. Inclusion criteria were composed as: metabolomic study of breast cancer only, in-vivo, in-vitro, clinical trial, and qualitative and quantitative analysis, published between 01/01/2010 to 31/01/2021, full-text articles, English written. Exclusion criteria were composed as: no association between BC and metabolome, metabolomic study of other cancers, review articles, conference proceedings, survey reports, comments, notes, or unpublished data, articles that do not met inclusion criteria.

Eligibility

In the stage of eligibility, studies were assessed using full-text articles. More specified information was extracted to decide the eligibility status. The data were narrowed down into the type of biological samples, type, and stage of BC, metabolites produced, metabolic pathways, as well as analytical and chemometric methods involved in determining the metabolites. The

articles were screened by the first author for relevance and reviewed by supervisor and co-supervisor. Subsequently, the non-eligible papers were excluded based on exclusion criteria: inadequate description on research methodology, focused on exogenous metabolites, no association between BC and metabolome, or ambiguous findings.

Inclusion

The final stage of systematic review was the inclusion of the eligible studies for data analysis. Previously collected data were summarized and presented in structured manner in the form of table, figure, or graph formats. The data were further divided based on the type of study (clinical or cellular), highest frequency metabolites, significant metabolic pathway, as well as summary of metabolomic study of BC.

RESULTS

In total, 924 articles were identified through the stated search strategy, and 866 remained after removal of duplicates. After screening, the titles, and abstracts of 137 references were selected, using the stated inclusion and exclusion criteria, as potentially eligible studies. Finally, 51 articles were included in which further sub-divided into 32 clinical research, 18 cellular research, and 1 clinical and cellular research.

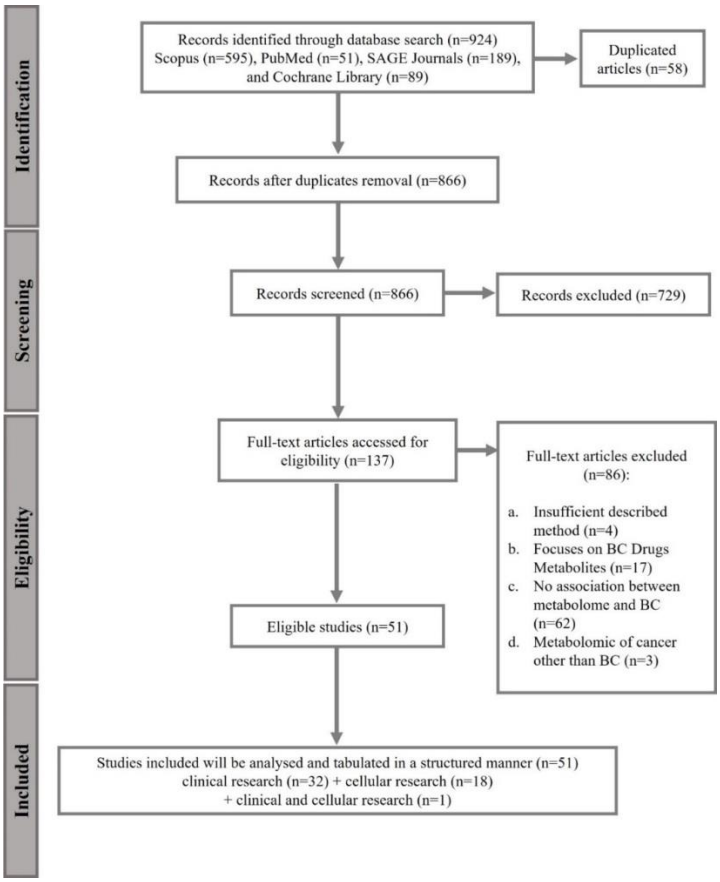

Figure 1: Flowchart of literature search

Clinical and Cellular Research

A total of 33 articles from clinical research and 19 from cellular research met the inclusion criteria in the final analysis, among which 14 studies were conducted with tissue, 13 with serum, 6 with plasma, 5 with urine, and 1 with breath while 6 studies reported MDA-MB-231 and MCF-7, 5 with MCF-10A, and 2 with BT-474 (Figure 2A). MCF-10A, mentioned in 5 articles, was the most frequently used normal human breast cell lines in research. Whereas, both MDA-MB-231 and MCF-7 were adopted as the most commonly studied BC cell lines, followed by BT-474 in 2 studies. Clinical metabolomics studies based on mass-spectrometry accounted for 21 studies, while 9 studies adopted NMR (Figure 2B). Four studies identified the metabolites by NMR, and the other 8 all adopted mass-spectrometry based

metabolomics for cellular research. Twenty studies were mainly targeted on specific metabolomes while the other ten were untargeted (Figure 2C).

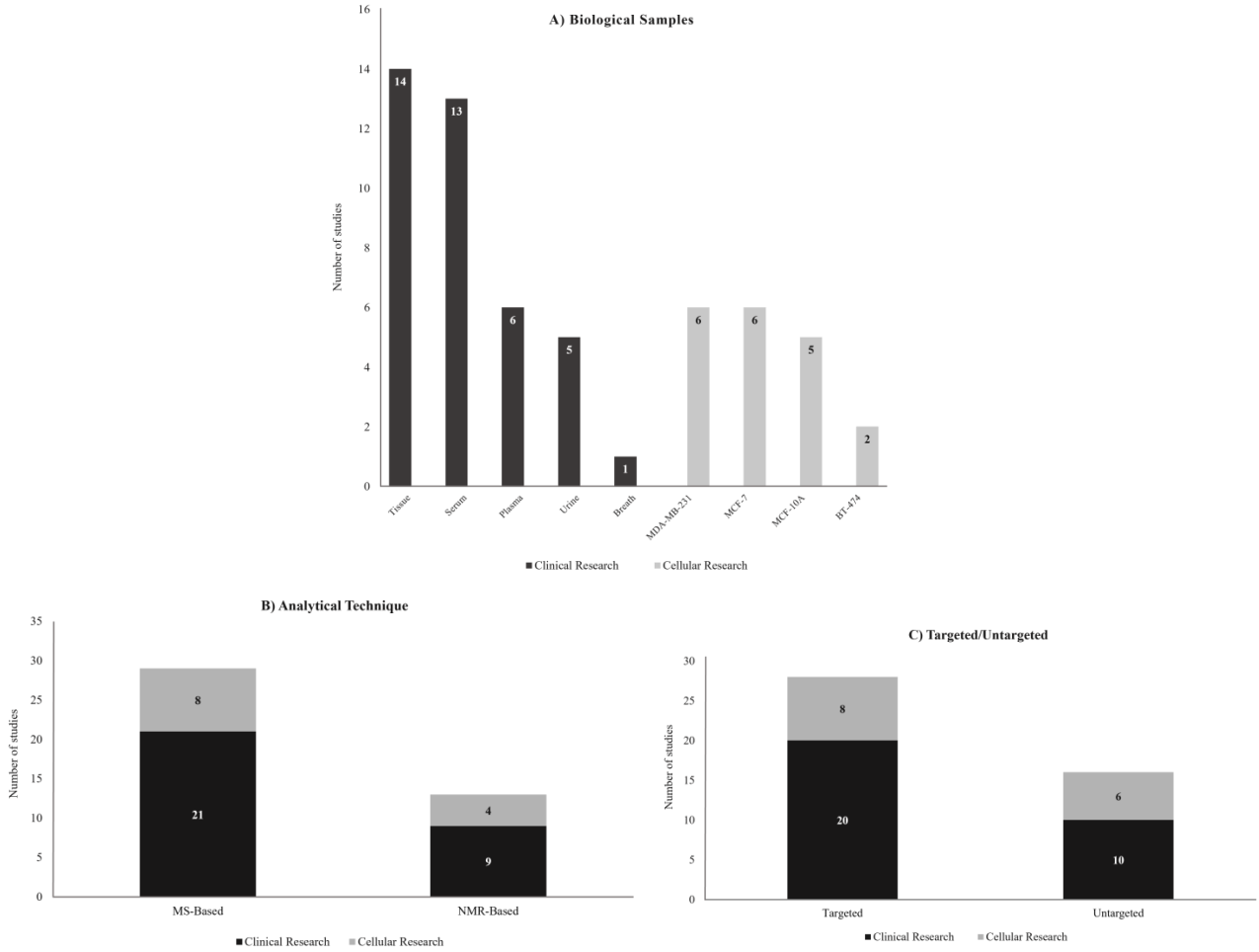

Figure 2: Characteristics of clinical and cellular research

Six cellular based metabolomic studies were mainly targeted on specific metabolomes while the other eight were untargeted. In these diagnosis-related studies, 21 high-frequency metabolites (reported in ≥ 3 studies) were recorded (Table 1). Glutamate with 10 hits in total has the highest frequency followed by glutamine at 9 hits and lactate at 8 hits. Results showed that BC patients exhibited at least one up- or down-regulated metabolite from amongst 21 high-frequency metabolites quantified in various biological samples. For cellular studies, 4 high-frequency metabolites (reported in ≥ 3 studies). Notably, glutamate, glutamine, and lactate were upregulated in all three studies except for glucose: one upregulated and two downregulated.

Table 1: High frequency metabolites in BC

No.	Metabolites	Type of Studies	Hits (Number of articles)	Changing Direction in BC	
				Up	Down

1	**Glutamate**	Clinical	10	Plasma (2)(11,32) Serum (2)(16,17) Tissue (3)(12,25,26)	Serum (2)(22,23) Tissue (1)(24)
		Cellular	3	Cell lines (2)(27–29)	-
2	**Glutamine**	Clinical	9	Plasma (1)(13) Serum (2)(16,23) Tissue (2)(34)	Plasma (1)(32) Serum (1)(17) Tissue (2)(12,14)
		Cellular	3	Cell lines (3)(27–29)	-
3	**Lactate**	Clinical	8	Serum (4)(15–18)	Serum (1)(23)
		Cellular	3	Cell lines (3)(27,28,39)	-
4	**Glucose**	Clinical	7	Serum (3)(22,23,38) Tissue (1)(14)	Serum (3)(15–17)
		Cellular	3	Cell lines (1)(28)	Cell lines (2)(37,39)
5	**Choline**	Clinical	7	Serum (3)(7,18,38) Tissue (4)(14,21,26,34)	-
6	**Creatine**	Clinical	7	Serum (4)(15,17,19,43) Tissue (1)(19)	Tissue (1)(26) Urine (1)(30)
7	**Tyrosine**	Clinical	7	Serum (2)(17,19) Tissue (2)(19,26) Blood (1)(38)	Serum (2)(16,23)
8	**Glycine**	Clinical	5	Serum (2)(16,17) Tissue (2)(21,26)	Urine (1)(30)
9	**Isoleucine**	Clinical	5	Serum (3)(16,17,43) Blood (1)(38) Tissue (1)(21)	-
10	**Phenylalanine**	Clinical	4	Serum (2)(17,38) Blood (1)(38)	Serum (1)(22)
11	**Glycerophos-phocholine**	Clinical	4	Tissue (2)(14,21,26) Blood (1)(38)	-
12	**Alanine**	Clinical	4	Serum (1)(17)	Plasma (2)(13,16) Serum (1)(23)

13	**Histidine**	Clinical	3	Serum (2)(17,43)	Serum (1)(18)
14	**Leucine**	Clinical	3	Serum (2)(16,17) Tissue (1)(21)	-
15	**Citrate**	Clinical	3	Serum (1)(17,23) Blood (1)(38)	-
16	**Phosphocholine**	Clinical	3	Tissue (3)(14,26,34)	-
17	**Taurine**	Clinical	3	Plasma (2)(32,42) Tissue (1)(26)	-
18	**Proline**	Clinical	3	Plasma (1)(13) Serum (1)(17)	Serum (1)(18)
19	**Pyruvate**	Clinical	3	-	Serum (2)(16,43) Tissue (1)(11)
20	**Valine**	Clinical	3	Serum (3)(7,41,43)	-
21	**Acetoacetate**	Clinical	3	Serum (1)(23) Blood (1)(38)	Serum (1)(16)

Analytical Approaches

A general overview of the most common analytical instrumentation used in BC metabolomics was illustrated in figure 3A. In total, 30 studies conducted the metabolomic analysis of BC using MS-based approach while 12 adopted NMR. Figure 3B categorized four MS-based techniques used in the identified metabolomic study of BC and one NMR-based approach. Gas chromatography mass spectrometry (GC-MS) was the most frequently reported with 25% of the studies applied this analytical approach and then closely followed by liquid chromatography mass spectrometry (LC-MS) with 21%, as well as tandem mass spectrometry (MS-MS) and time of flight mass spectrometry (TOF-MS) at 11% respectively. Overall, as compared to other analytical techniques, MS-based approach accounted for almost 70% (30 out of 44) of the identified studies. In all, thirteen studies performed NMR-based metabolomics on BC, among which 6 studies analyzed serum samples, 4 with cell lines and tissues, 2 with plasma, and 1 with urine (Figure 3C).

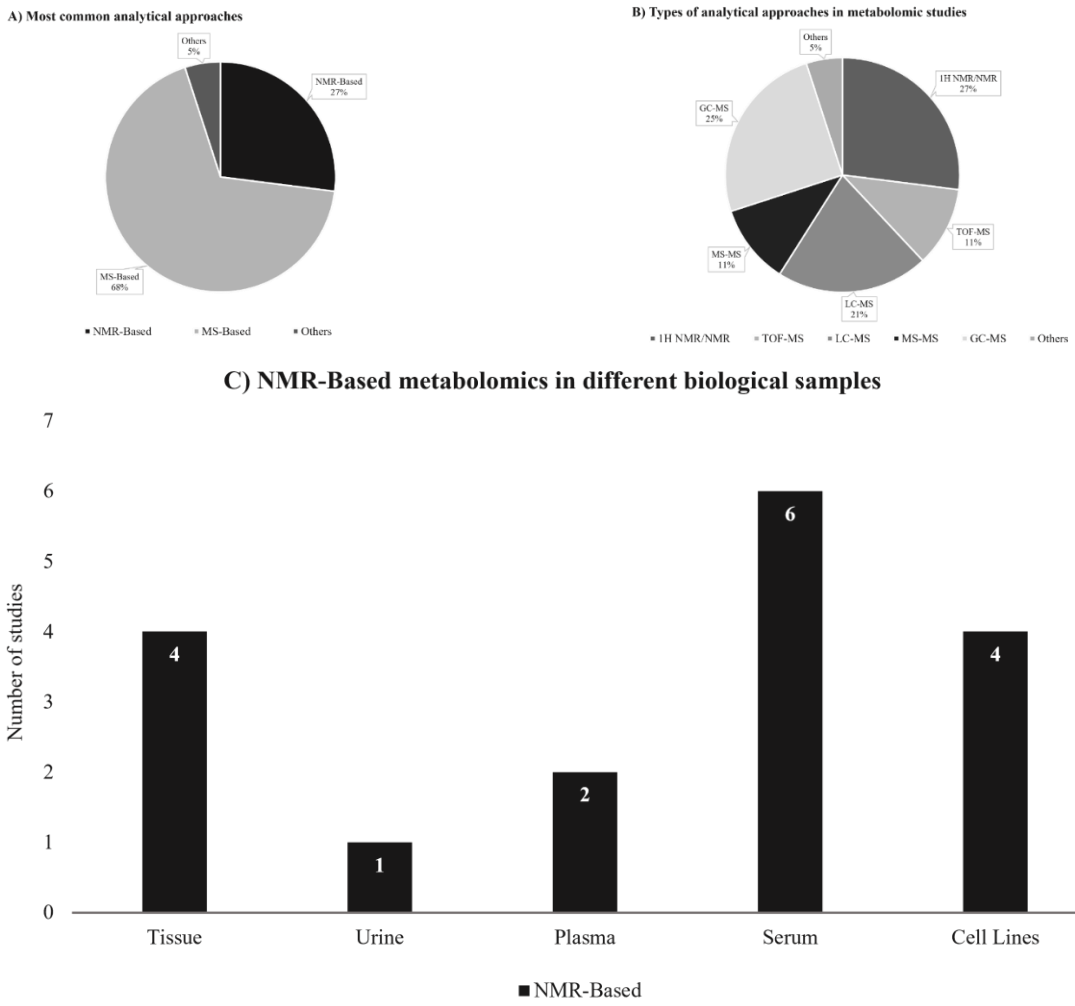

Figure 3: Summary of analytical approaches in BC metabolomic studies

Main Chemometric Methods

In total, more than half (59%) of the identified metabolomic studies reported principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA) as the main chemometric methods used in the interpretation and analysis of BC metabolomic data. Two studies incorporated all three statistical techniques, nine used a combination of two (e.g., PLS-DA and PCA), and fourteen used only one type of chemometric method.

DISCUSSION

In this review, we performed a systematic analysis of cellular and clinical metabolomic studies on BC diagnosis. As a result, a series of potential biomarkers were summarized and reported. A total of 21 high-frequency metabolites in clinical research (reported in ≥3 studies) were listed, and some metabolic biomarkers (e.g., glutamate, glutamine, and glutaminase (GLS)) showed changing trends with glutaminolysis as the most significant pathway involved in the regulation of glutamate and glutamine, both in clinical and cellular studies (11–14).

Based on cellular and clinical studies, we found that some significant metabolites (e.g., glutamate, glutamine, lactate, choline, leucine, creatine, histidine, and taurine) were repeatedly reported (15–18). Glutamine, histidine, tyrosine, and creatine showed significant changes in tissue (19–21). Glutamate and glutamine demonstrated the highest frequency of detection with 10 and 9 hits respectively, indicating the potential as a sensitive biomarker for BC diagnosis (16,17,30,22–29). Despite the inconsistent changing trend of both metabolites among the studies, majority of the studies acknowledged the significant elevation of glutamate influx was due to the increased glutamine catabolism (31–34).

According to Bhowmik et al., (2015), more than 1.5-fold accumulation of glutamate and glutamine was observed in cell lines expressing epithelial-mesenchymal transition (EMT) transcription factors (27). Both were substrate and product of glutaminolysis. This finding was further supported with reports by Cao et al., (2014) and Budczies et al., (2015) in which elevated levels of glutamate were associated with aggressive breast cancer subtypes (35,36). As for lactate, Bhowmik et al., (2015) demonstrated ≥ 3.5-fold increase (27). On the contrary, limited availability of glucose could result in reduced glycolysis which correlated with tumor aggressiveness by increasing stem-cell-like cells (SCLC) cell populations (22,37,38).

Elevated lactate level, on the other hand, was described as one of the early findings of metabolic changes reported for BC (18,29,39). Lactate was known to be altered in the metastatic stage (40). The increased level was well correlated with Warburg effect as well as high demand for cellular proliferation (increased glycolytic activity resulted in more lactate production) (15). The high fold changes of lactate were associated with tumor cells' survival in which they increased the energy consumption by lactate production (Warburg effect) (20).

Among the 21 metabolites, choline, isoleucine, glycerophosphocholine, leucine, taurine, and valine demonstrated consistent increasing trends [41–43]. Choline, in particular, was one of the most prominent metabolites in which it could differentiate BC from tissues or normal cells (18,21,44). Choline was also invariably associated with increased proliferation of tumor cells in BC (14,18). It was also characterized as the signs for BC recurrence (40). Choline pathway specifically showed a significant increase of choline levels in BC tissues samples as compared to normal breast tissue samples (34).

Glycerophosphocholine, a choline-containing metabolite also shared the same increasing trend specifically in ER-negative BC (26). Isoleucine on the other hand was shown to be upregulated in metastatic BC as compared to early BC(43). It was characterized with higher risk metabolomics profile together with glutamate and leucine. Leucine was characterized with higher risk metabolomics profile with no significant correlation with tumor stage (43). Citrate was elevated in serum TNBC samples (23). The increase was seen between the early BC and metastatic BC (45).

Higher content of taurine suggested increase bioenergetics of tumor cells. Taurine showed antioxidant activity were increased in plasma from cancer subjects could be involved in protecting cancer cells from excessive damage by oxidative stress (42). In addition, the elevated content in taurine is also suggestive of increased utilization of the amino acid methionine, essential for the synthesis of methyl group donor compounds, the amino acid cysteine, and the antioxidant glutathione (46). Taurine was also found to be significantly elevated in the blood of BC patient which reflected its correlation with risk, response, and survival rate of cancer, as well as with the oncometabolite fumarate (32). It also correlated with the up regulation of arginine methyltransferase activity. ER-positive samples had higher levels of taurine (26).

The alteration in glutamate and glutamine associated with the glutaminolysis pathway could be considered as another important hallmark in tumor metabolism besides the “Warburg effect” (12–15,31,32). According to Lampa *et al.,* (2017), glutaminolysis was a vital step since it was rapidly consumed by cancer cells to fulfill its overwhelming metabolic demand (47). The intracellular processing of glutamine into glutamate by the enzyme glutaminase (GLS) followed with subsequent deamination into α-ketoglutarate (αKG), the intermediate of citric acid cycle served as an important source of nitrogen, carbon, and energy in tumor cells (11,47).

Pathway analysis showed that glutaminase enzyme (GLS) played crucial role in the BC progression. Shah & Chen, (2020) emphasized that GLS overexpression allowed the increase of glutaminolysis metabolism, thus providing a means for the cancer cells to produce molecules needed for anabolic growth as well as replenish the tricarboxylic acid (TCA) cycle (31). The finding was further supported by Lampa *et al*., (2017) in which this study reported the significance of GLS gene in the survival and growth of TNBC tumors *in vivo* and *in vitro* (47). It was confirmed by the enhanced utilization of glutamine, high GLS to glutamine synthetase (GLUL) ratio, and increased glutamate to glutamine ratio.

The key enzyme for glutamine metabolism was glutaminase isoenzymes GLS1 and GLS2. Interestingly, both isoenzymes have contrasting functions in cancer formation (48–51). The well-established glutaminolysis dependence in various types of cancers were linked to GLS1 isoforms. In particular, the GLS1 upregulation was correlated with increase tumorigenesis, while GLS2 expression was often related to tumor-suppressing activity (51).

According to Dias *et al*., (2020), the significant elevation

in the GLS level in BC patients might be indicative of the oncogenic or anti-oncogenic condition (49). High GLS1 in TNBCs was correlated with poor prognosis and patients were dependent on the GLS activity and exogenous glutamine for survival (51). On the contrary, GLS2 upregulation in tumor cells promoted antiproliferative response with decreased formation of tumor cell colony in hepatocellular carcinoma (HCC) as well as cell cycle arrested at the G2/M phase (52). In few cell lines studies, the knockdown of GLS2 reduced glutamine-linked metabolic phenotypes and decreased cell proliferation (49,51). Of concern, GLS2 overexpression or amplification was correlated to an overall, metastasis and disease-free survival in BC in which could be utilized as prognostic BC biomarker.

Along with the heterogeneity of BC, metabolites in different patients could vary based on different samples. Overall, majority of the metabolites reported were obtained from serum and tissues samples (14–16,18,20). A review by Stevens *et al*., (2019) reported that serum and plasma were the most commonly used blood fractions in metabolomic studies (53). Serum, in particular, has a higher level of metabolites (e.g., protein fragments and some peptides) as compared to plasma (54–56). Both Paglia *et al*., (2018), who utilized targeted approach, and Nishiumi *et al*., (2018) who utilized untargeted approach, suggested serum as biological samples with higher sensitivity (55,56). Analysis of tissues was equally important, as the tissues were the hub of metabolic turnover for diseases (57). By contrast, cellular studies have an advantage of avoiding the heterogeneity resulting from diverse samples. However, metabolites detected could also be fluctuant, which is induced by the incubation time and pH of mediums (4).

Gas chromatography mass spectrometry (GC-MS) has been acknowledged as the gold standard in metabolomics study (58,59). Generally, GC–MS was considered a versatile analytical technique (60). This was because of its excellent separation capability, reproducibility, sensitivity, selectivity as well as robustness (59,61,62). In comparison with LC-MS, GC-MS achieved better separation of metabolite and could generally avoid suppression of ion due to its MS ionization nature and the use of the gaseous phase for analysis (63). Somehow, the fact that this technique could only detect volatile compounds, as well as limited to ionizable metabolites served as inherent limitations for GC-MS (58).

Tandem mass spectrometry (MS-MS) on the other hand could increase the precision and selectivity of compounds quantifications and identifications by providing additional information for comparison with databanks and structural elucidation (64). Instead of configuring with only one analyzer, two or more mass analyzers were possible in tandem mass spectrometers (MS/MS), allowing great utility including quantitative analysis, characterization of complex molecules, and compound identification (65). A study by Weiss & Schilsky, (2019) reported a recent metabolomic study that applied tandem mass spectrometry (MS-MS) on the identification of congenital disorders in newborns (66). MS-MS has markedly expanded the screening ability for 50 metabolic diseases by detecting the presence of specific metabolome in a single dried blood spot (DBS). Collectively, this study confirmed the practicality of using MS-MS in specific screening of Wilson disease in newborn.

The wide applications of NMR in different samples including gas, liquid, and solid were explained by Emwas, (2015) and Kruk *et al*., (2017), in which NMR was mainly used in structural elucidation and molecular identification, as well as in the study of chemical and physical properties of molecules (e.g., molecular dynamics and electron density) (61,67). High-resolution magic-angle spinning (HRMAS) is fairly recently developed technique in NMR spectroscopy, whereby the applications were not only restricted to liquid and solid samples but also extended to intact tissue samples. Comparable resolution spectra could be obtained by spinning the tissue sample at a “magic angle” of 54.74° at high speed. HRMAS specifically allows spontaneous detection of tissue’s chemical composition with no pre-preparation steps required. In NMR-based metabolomics study, these methods provided correlation between bio-fluids metabolic profiles and specific histology of tissue samples. Hence, HRMAS NMR spectroscopy has been widely applied in metabolomic study of small intact tissues samples including kidney, brain, liver, as well as breast tissue.

Choi *et al*. (2017) has reported a study to identify the correlations between metabolic profiles of core needle biopsy (CNB) specimens and the currently used molecular markers in patients with ER-positive BC by utilizing HRMAS magnetic resonance spectroscopy (HRMAS MRS) method (21). The metabolic profiles were compared according to HER2 and Ki-67 (proliferation index) status. Tumors overexpressing HER2 have been found to be more aggressive with a high rate of recurrence and mortality, as this receptor plays a major role in promoting the growth of cancer cells (68). This further explains HER2 status remains

clinical importance as prognostic and predictive biomarkers especially for HER2-targeted therapies (69,70). In regard to OPLS-DA score and loading S-plots analyses of the HRMAS MRS spectra for HER2 status, high level of glutamate and glycine were detected in HER2-positive group when compared to HER2-negative[54].

The aforementioned findings indicate that HER2-positive BC had the highest glutamine metabolism activity in comparison to its counterpart (HER-negative). A similar observation was made whereby the high Ki-67 group demonstrated higher levels of glutamate than the low Ki-67 group. It is important to note that Ki-67 is present in cells that are actively growing and dividing, which justifies it is commonly used as a proliferation marker in breast cancer (71). Breast cancer patients whose categorized under high Ki-67 group have a larger number of proliferating cells and are commonly associated with worse prognoses and worse survival rates (72). A noticeable increase in glutamate level in the high Ki-67 group, perhaps reflecting the greatest increase in glutamine metabolism (73). Considering the above-mentioned evidences, HRMAS MRS allows for direct measurement of non-liquid tissue and provide simultaneous insights of the chemical pathology relating to BC.

Nevertheless, the primary limitation of NMR is linked with low sensitivity (61). Although important signal enhancement utilizing higher cryo-probes, digital signal processing, and magnetic fields improved the sensitivity, today's NMR technology still cannot detect many low-abundances metabolites. For instances, only hundreds out of thousands detectable or measurable metabolites in biofluids reported as reliably detected by NMR (74–76). While metabolites with high abundance were frequently important, low abundance metabolites were similarly crucial for biomarkers diagnostic purposes.

Furthermore, the overlapping resonances represented by proton NMR in metabolites quantification and identification in biofluids remained as another continuing challenges (61,77). In particular, the used of proton NMR in metabolomics studies produced narrow chemical shift dispersion and majority of the resonances were found in between 1 to 4 ppm. Significant challenges happened in compounds and peak assignment, especially at lower strengths magnetic field. Specifically, the spectral overlap problem and peak assignment's reliability could be improved via 2-dimensional NMR (77). Somehow, despite the improved processing and signal acquisition techniques in 2D NMR, strong inertia remained exist which limits the applicability in metabolomic studies.

Additionally, NMR-based techniques were expensive as compared to MS-based or many other frequently used analytical approaches (61,78). Besides, NMR instruments required substantial laboratory space isolated from radio and magnetic interference, nonvibrational floors, as well as highly skilled operators to handle the machines (61). These overriding issues have made it challenging in expanding NMR used in metabolomics fields.

As evidenced by the predominant specificities and sensitivities in previous studies, metabolomics has shown advantages in the early diagnosis of BC (4). The use of metabolomics leads to the generation of a significant amount of data. With current high field spectrometer technology, visual analysis of NMR and MS is not an efficient means of interpreting biofluid spectra, because the high level of metabolic information represents a significant analytic challenge (61,79,80). Therefore, as with other analytic platforms generating multivariate data (e.g., proteomics, genomics), automatic data reduction and chemometric approaches were used to enable efficient mining and extraction of information from large spectral metabolomic databases (80).

Silva *et al*., (2019) in their urinary metabolomics study of BC, applied principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA) to the metabolites dataset to give insights on the group's separations (i.e., 38 healthy controls and 40 BC patients) (30). As an unsupervised method, PCA was conducted to visualize the differences/similarities of the urine sample profiles between the groups. Particularly, samples were individually analyzed with no classification. The results observed a tendency of clusters formation between the groups explained by total variance of 54.6%. Next, PLS-DA, a supervised method was applied to maximize the groups separation. Ten significant metabolites were identified: glutamine, glycine, creatine, trimethylamine, dimethylamine, serine, mannitol, α-hydroxyisobutyrate, trigonelline, and cis-aconitate (19). Then, OPLS-DA was applied to further maximize the BC and control groups separation by demonstrating the variable responsible for discrimination. As a result, a good separation was obtained with total variance of 54.8 % and average prediction accuracy more than 90%. Overall, the distinguished metabolomic patterns demonstrated a unique metabolite profile for each group.

CONCLUSION

In conclusion, metabolomics enables in-depth identification of BC metabolic profile. Numerous studies have demonstrated potential metabolites for BC diagnosis. This review had systematically identified, screened, and analyzed 51 articles related to metabolomic of BC reported between 1st January 2010 to 31st January 2021. Those articles were successfully classified based on the type of research, biological samples, analytical techniques, and main chemometric methods. A total of 21 high-frequency metabolites were studied for their prevalence in BC. Metabolite's alterations involving glutamate, glutamine, lactate, choline, and taurine provide evidence of tumorigenesis. Glutaminolysis presented as the most significant pathway with GLS2 as potential biomarker for BC diagnosis. MS-based metabolomics provides an excellent approach as the most common analytical instrumentation used offering a combined selectivity and sensitivity platform for metabolomics study. PCA, PLS-DA, and OPLS-DA were significantly reported as the chemometric methods used in interpreting metabolomic databases. Even though there were some inconsistency and conflicts in these current studies, metabolomics still serves as great potential in early tumor stage identification in the future. The relative non-invasion and advantages of convenience in comparison with tissue biopsy and imaging screening, considered metabolomics as a relevant tool in early BC diagnosis. Collectively, the metabolomic of BC is still at an early stage, however it may lay foundation for understanding the progression and development of BC for BC treatment.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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