Studies on the Methods of Diagnosis and Biomarkersused in Early Detection of Breast Cancer in the Kingdom of Saudi Arabia: an Overview

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Abstract: Breast cancer (BC) affects women of all socioeconomic levels in both developed and developing countries. The global annual mortality is expected to be close to 500,000 women. The most traditional and time again used symptoms and diagnostic methods do not fully accommodate the intricacies that warrant early detection of BC. If detected early, BC is more easily treated and often curable, hence; world-wide efforts have been focused in this direction and the Kingdom of Saudi Arabia (KSA) is not lagging behind. The present review is an attempt to project the different procedures undertaken for early detection and diagnosis of BC as revealed by papers published in peer reviewed English language articles cited in Pub Med, Pub Med Central, Science Direct, Up-to-date, Med Line, Comprehensive data bases, Cochrane library and the internet (Google, Yahoo). The methods used in the diagnosis and detection of BC in the KSA include mammography, ultrasound, biopsy (fine needle aspiration and surgical biopsy), computerized tomography, positron emission tomography (PET), histopathology, cytology and sentinel lymph node biopsy, in addition to advanced research on molecular markers. Although there has been considerable improvement in researches on biomarkers, there is still a lot to be done in comparison with global achievements. The review will also discuss the lacunae in diagnostic endeavors, which will go a long way to abridge the rampant mortality due to BC.

Key words: Breast Cancer ⋅ Diagnosis ⋅ Early Detection ⋅ Biological Markers

INTRODUCTION

Breast cancer is becoming an increasingly significant public health threat throughout the world. It affects women of all socioeconomic levels in both developed and developing countries [1]. Globally, almost 1.4 million women are diagnosed with BC in 2008 and about 459,000 deaths are recorded. The incidence was much higher in developed countries compared to less developed countries [2].

The most traditional and time and again used symptoms to detect BC are presence of a hard lump or thickening in the breast or in the armpit, uneven edges, swelling, dimpling, redness, or soreness of skin, change in shape or appearance of the nipple and nipple discharge. These primitive symptoms are approved by American Cancer Society and warrants consultation, whenever they persist [3]. In a study on predisposing factors for breast cancer, Al-Amoudi and Abduljabbar [4] found breast mass as the most common symptom, followed by change in the size of breast and discomfort.

Efforts have been focused on early detection since BC is more easily treated and often curable if it is detected early. Three tools for early detection of breast cancer are regular breast self-examination (BSE), clinical breast examination (CBE) by a medical professional and screening mammography in patients who are at risk of developing the disease [5,6]. Generally, palpable breast

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mass is a common problem in female patients. The diagnostic delays of BC occur due to generally low index of suspicion. Nevertheless, as the cancer grows, symptoms begin to appear [7].

Any bothersome changes or symptoms in breast are supposed to be shown to a competent medical professional, who may perform a thorough Physical examination. In addition, investigations are often suggested to evaluate the condition. These include X-ray mammography, which can help to ascertain the breast mass. An ultrasound can show whether the lump is hard or fluid-filled. Needle biopsy of a breast lump is required to establish if the lump contains fluid. The fluid can be aspirated and sent to laboratory for further analysis. On preliminary diagnosis of BC a surgical biopsy or breast lump removal is carried out to provide a slice or the entire breast lump for laboratory study. When the BC is diagnosed, other tests including scans and blood tests are performed to check if the cancer has already spread to adjacent or distant parts. This way, the stage of the disease can be determined. Depending on the stage of disease, mastectomy, radiation therapy, chemotherapy, hormonal Therapy, biologically targeted therapy, or a combination of these may then be recommended. In most cases, if the cancer is detected early and treated appropriately, BC patients can usually lead a cancer free life.

The objective of this review is to analyze the different procedures commonly used for the early diagnosis of BC in the KSA. These include mammography, ultrasound, biopsy (fine needle aspiration, surgical biopsy), computerized tomography, positron emission tomography (PET), histopathology, cytology and sentinel lymph node biopsy. These methods are the bases for advanced research on molecular markers. The present review has also recorded flaws in some methods and the need for improvement in researches on biomarkers as compared to the global achievements.

Methodology: The present review adopted on the diagnosis and markers used in early detection of BC in the KSA have included literatures survey. The selected Publications have comprised different procedures which are commonly used in the KSA for the early diagnosis of BC. These include mammography, ultrasound, biopsy (fine needle aspiration, surgical biopsy), computerized tomography, Positron Emission Tomography (PET), Histopathology, cytology and sentinel lymph node biopsy, in addition to the experimental and practical use of different molecular markers. The task was met up with

peer reviewed English language articles cited in Pub Med, Pub Med Central, Science Direct, Up-to-date, Med Line, Comprehensive data bases, Cochrane library and the internal (Google, Yahoo). The strategy of search combined terms that included the title and the keywords.

Review of Literature: The present study is a systematic review of literature on different procedures used more commonly in the KSA for the early diagnosis of BC. These include mammography, ultrasound, biopsy (fine needle aspiration, surgical biopsy), computerized tomography, positron emission tomography (PET), histopathology, cytology and sentinel lymph node biopsy, in addition to the experimental and practical use of different molecular markers. Also discussed are some deficits, flaws and possible improvements as compared to the global literature, so that the BC-related mortality can be abridged in the KSA.

Mammograms: Mammograms are the best method to detect BC early when it is easier to treat and before it is big enough to feel or cause symptoms. Having regular mammograms can lower the risk of dying from BC. The first national public BC screening program in Saudi Arabia conducted BC screening by mammograms using the breast imaging-reporting data system and determined correlations between imaging findings, risk factors and pathological findings [8]. Among the different imaging methods, mammography is the most operative to detect early-stage BC. However, understanding the data in images is crucial to develop a model that fits well. Statistical distributions are widely used in modeling of the data. The estimation of thresholds is based on the statistical parameters of the histogram and the results on mammography images show improvement in the accuracy of detection [9]. Breast imaging has made gigantic advances in the recent past. Many novel methods are being used in detecting distant metastasis, recurrent disease and assessing response to treatment. Full-field digital mammography augments the background of the lesion disparity and gives better sensitivity, which makes it possible to see through the dense tissues by altering computer windows. This may be particularly useful in women with dense breasts [10].

A mammography program conducted in Al-Qassim (Saudi Arabia) on 1628 women, showed the cancer detection rate to be 0.24 per cent out of 1.5 per cent biopsies performed. Most of the performed indicators were not available and many of the available indicators did not meet international standards [11]. Bilateral BC is

invariably advanced when diagnosed, however; mammogram is a valuable tool in early detection. Whether synchronous or metachronous, both breasts often share the same histological type [12].

Fine Needle Aspiration (FNA): Fine needle aspiration breast biopsy is an efficient tool and yields a definitive diagnosis and its use for routine diagnosis is greatly encouraged. In a study to determine the diagnostic efficacy of FNA, Mansoor and Jamal [13]used 72 cases that had both FNA cytology and subsequent histology diagnosis and found the sensitivity and specificity of FNA procedure to be 98.4% and 60%, respectively, while the overall diagnostic accuracy was 93%.The authors concluded FNA breast biopsy as an efficient tool which yields a definitive diagnosis as it has high positive (93.9%) and negative (85.7%) predictive values.

Fine-needle aspiration cytology (FNAC) is a widely practiced technique in the diagnosis of breast carcinoma and it is performed before definitive treatment, at most institutions. Khan *et al*. [14] reviewed 125 cases of breast carcinoma, which were primarily diagnosed by FNAC, with subsequent confirmation by histopathology. The authors devised a simple system for grading breast carcinoma, based on six cytological features including cellular pleomorphism, nuclear size, nuclear margin, nucleoli, naked tumor nuclei and mitoses for grading. This scoring system was used, to classify ductal carcinoma into three cytological grades, which showed close correlation with the established histological grades. The other additional features included were the presence or absence of necrosis and stromal invasion, smear cellularity, degree of cell dispersion or clustering, lymphoplasmacytic infiltrate, presence of tubular structures, cytoplasmic appearance of the tumor cells and smear background. These additional parameters were found helpful in assigning the correct grade, in cases with borderline scores.

FNA findings have been rarely reported in Sarcomatoid carcinoma of the breast, which is a very uncommon neoplasm. Straath of *et al*. [15] reported a case of sarcomatoid carcinoma of the breast that was diagnosed as a typical ductal carcinoma by cytology. In a 45-year old BC patient, the FNA smears showed extensive metachromatic stroma of the DIFF QUICK-stained smears. The findings in this case suggested that sarcomatoid carcinoma of the breast is often overlooked or misdiagnosed due to subtlety of the stroma or the predominance of the mesenchymal component. The authors found that use of DIFF QUICK stain is very

helpful in the identification of the stroma in this neoplasm. For the diagnosis of fibroadenoma also, FNA has been found as a highly sensitive method [16]. FNA has proven to be a reliable test in differentiating between phyllodes tumor and FA with high sensitivity and good reproducibility, however; adequate training and continuing education is of paramount significance [17].

A 35 year old patient presented with right-sided breast lump associated with hepato-splenomegaly was diagnosed for polymorphous lymphoma (consisting of medium to large-size cells with immature chromatin), upon fine-needle aspiration biopsy (FNAB). Flow cytometricimmunopheno typing showed expression of CD2, CD3 and CD7. This case indicated that gamma/delta peripheral T-cell lymphoma can be diagnosed by FNAB. Cytogenetic analysis showed 48XX+i7 (q11.2), +7(3) [18].

Use of Methylene Blue Dye Facilitates Surgical Identification: In a study on 18 non-palpable breast lesions (14 parenchymal and 4 ductal lesions) detected by mammography, Makanjuola *et al*. [19] used Kopan's localization needle guided by an alpha-numerical plate in the parenchymal lesions and ductography with methylene blue dye for the ductal lesions was used. Three out of 14 were positive for invasive cancer, while the rest showed ductal epithelial hyperplasia and fibrocytosis. The ductal lesions were localized correctly and pathologically confirmed as papillomata. The authors concluded that localization of intraductal lesions with methylene blue dye facilitates surgical identification.

Positron Emission Tomography (PET): A PET scan offers the advantage of screening the entire body, excluding the presence of metastases. The sensitivity, specificity and accuracy of PET scans were conducted in 109 patients having primary recurrent or metastatic BC. The patients had a PET scan, X-ray or CT scan of the chest, an ultrasound or CT scan of the liver and a bone scan. Mammography was available for 86 patients. Analysis of correlation showed PET scanning as the only non-invasive imaging procedure that will detect tumors in the breast, lymph nodes, lung, liver, bone and bone marrow with high sensitivity, specificity and accuracy. It is a valuable tool in the management of patients in all stages of BC for diagnosis, staging and following treatment response [20].

Bakheet *et al*. [21] reported primary breast sarcoma in three patients who showed intense 2- [18F]-fluoro-2-deoxy-D-glucose (F-18 FDG) breast uptake on the whole-body scan. In two patients, the uptake was not regular

and had hypodense lesions noted on the chest CT which demonstrated areas of tumor necrosis. The F-18 FDG whole-body PET scanning accurately staged the tumors in these two patients and documented local recurrence in the third patient. There was evidence of a high-grade sarcoma, a primary rhabdomyosarcoma and malignant cystosarcomaphyllodes of the breast, as revealed by histopathology. Thus F-18 FDG whole-body PET imaging can be useful in diagnosing and staging primary breast sarcomas, similar to breast carcinoma.

F-18 FDG-PET was performed on a 50-year-old woman, who had an irregular, mobile, firm right breast mass that became progressively larger (18 x 15 cm) within 3 months. The FDG-PET showed a ring-shaped breast uptake consisting of high peripheral glycolytic activity and a cold center, perhaps, representing necrosis or hemorrhage. Evidence of lymph node involvement or distant metastases was absent in the whole-body images. The results were confirmed by computed tomography of the chest, abdomen and pelvis. The Cytological examination of a FNA of the breast mass showed diffuse large B-cell, intermediate grade, non-Hodgkin's lymphoma [22].

Cystic infiltrating ductal carcinoma of the breast is uncommon and frequently misdiagnosed because of the predominant cystic presentation clinically. Baslaim *et al*. [23] presented three premenopausal patients with huge cystic breast lesions. In the first patient, mammography showed a high-density, well-circumscribed huge breast mass, whereas in the other two patients mammography was not possible because of the huge breast size. Breast ultrasound in these cases showed large cystic lesions suggestive of tumor with central necrosis or bleeding from which a variable amount of bloody fluid was aspirated. A whole-body PET scan in these patients showed an intense focal 18-FDG breast uptake corresponding to the solid component and a ring like uptake corresponding to the cystic component most likely representing tumor necrosis, hemorrhage, or both. Furthermore, whole-body PET scan was valuable in predicting the response to chemotherapy, characterizing the pelvic abdominal mass and detecting the presence of hepatic and spinal metastases. 18-FDG PET scan can help characterize a cystic breast mass by identifying the extent of the cystic and the solid component. It is also useful in staging cystic infiltrating ductal carcinoma by detecting lymph node involvement as well as distant metastases.

Inflammatory breast cancer (IBC) is a locally advanced breast cancer. It is the most aggressive form of cancer, which can be diagnosed based on a clinical or pathologic basis. The usefulness of 18 FDG-PET scans was evaluated to diagnose and stage IBC [24]. The authors reviewed the medical records of seven IBC patients who underwent FDG-PET scanning for the initial staging. All patients presented with diffuse breast enlargement, redness and peaud'orange for 1 to 5 months' duration. The FDG-PET scan was found useful to display the pattern of FDG breast uptake that reflects the extent of the pathologic involvement in IBC (i.e., diffuse breast involvement and dermal lymphatic-spread). It was also suitable to detect the presence of lymph node and skeletal metastases, demarcating the extent of the disease locally as well as distally.

Biomarkers Used in the Detection of BC: Early detection of breast cancer reduces the agony and cost to society associated with the disease. A sensitive assay to identify biomarkers that can accurately diagnose the onset of BC using non-invasively collected clinical specimens is ideal for early detection.The targeted therapies in BC that include molecular protocol are sensitive and have higher efficacy than conventional therapy agents in the treatment of BC [25, 26]. The biomarker detection is used to identify and diagnosis, in addition to determining prognostic response to different modes of therapeutic regimens.

Proteins: The actin-bundling protein, fascin is a member of the cytoskeletal protein family which has restricted expression in normal cells, however; it is reported to be induced in various transformed cells including BC cells. Al-Alwan *et al*. [27] investigated fascin expression in BC cells and found the expression to establish a gene expression profile consistent with metastatic tumors and it can be used as a marker for BC detection.

B7-H1 is a protein that is encoded by the CD274 gene in humans. It is reported to increase the apoptosis of tumor-reactive T lymphocytes and reduces their immunogenicity. However, there has been no direct evidence associating the expression of B7-H1 with cancer in general and BC in particular remained unsolved till Ghebeh *et al*. [28] reported direct evidence linking B7-H1 with breast cancer in 22 of the 44 breast cancer specimens. The expression expressed by tumor-infiltrating lymphocytes (TIL) in 41% samples and was not restricted to the tumor epithelium in 34% of the samples. The intra-tumor expression of B7-H1 was significantly associated with histologic grade III-negative, estrogen receptor-negative and progesterone receptor-negative. The expression of B7-H1 in TIL was linked with large tumor

size, histologic grade III, positivity of Her2/neu status and severe tumor lymphocyte infiltration. These results demonstrated that B7-H1 may be a significant risk factor in breast cancer patients and may represent a potential immunotherapeutic target. In another study Ghebeh *et al.* [29] investigated the effect of proliferation, as measured by Ki-67 and mitotic count, on the induction of B7-H1. H and E stained sections were used to screen mitotic count in 69 breast cancer patients. A direct relation was observed between proliferation and the expression of B7-H1 in BC patients. The association between B7-H1 induction and cell proliferation was also investigated *in vitro*, in which a strong link was clearly demonstrated between B7-H1 expression and the presence of the proliferative Ki-67 marker.

Tristetraprolin (TTP) is a tandem CCCH zinc-finger RNA-binding protein that regulates the stability of certain AU-rich element (ARE) mRNAs. Reports in the literature suggested that TTP is deficient in cancer cells when compared with normal cell types. In a study on deficiency of TTP, Al-Souhibani *et al.* [30] reported that TTP, in a 3' untranslated region-and ARE-dependent manner, regulates an important subset of cancer-related genes that are involved in cellular growth, invasion and metastasis.

Nucleosides: 2'-Deoxycytidine (dCyd), a pyrimidine nucleoside found at elevated levels in the plasma of cancer patients is considered to be a biomarker for BC chemotherapy [31]. The concentration of dCyd in the sample was estimated by its ability to inhibit the binding of the antibody to the immobilized 5'sdCyd-BSA and subsequent color formation in the assay. The proposed Enzyme Immunoassay is expected to contribute in further evaluation of dCyd as a prognostic marker for breast cancer chemotherapy and elucidation of the role of dCyd in various biological and biochemical systems [32].

Hormone Receptors: The estrogen and progesterone receptors are known to fuel the growth of BC cells. Hence, the samples from all cases of BC are tested for presence of estrogen and progesterone receptors. Breast cancers that contain estrogen receptors are often referred to as "ER-positive" and those with progesterone receptors are "PR-positive." Hormone receptor-positive BC tends to grow more slowly and have a better outlook than cancers without these receptors. Cancers that have these receptors can be treated with hormone therapy such as tamoxifen or aromatase inhibitors. The growth of a large proportion of BCs is stimulated by estrogens, while, Progesterone plays a significant role in breast development and carcinogenesis [33].

Tamimi *et al.* [34] analyzed the molecular subtypes present in the Saudi population, using surrogate markers (ER, PR, HER2, EGFR and CK5/6) for gene expression profiling and classified 231 breast cancer specimens. The study incorporated correlation of these molecular classes with Ki-67 proliferation index, p53 mutation status, histologic type and grade of the tumor. A high Ki-67 proliferation index was noted in basal followed by HER2+ class. Overexpression of p53 was predominantly observed in HER2+ followed by the basal group of tumors. A strong correlation was noted between invasive lobular carcinoma and hormone receptor expression. The study suggested that the molecular analytic spectrum currently used may not completely cover all molecular classes and there is a need to further refine and develop the existing classification systems.

In a 9 year study the status of estrogen receptor/progesterone receptor (ER/PR) and human epidermal growth factor receptor 2 (HER2) was determined in 852 patients. The results demonstrated that ER/PR and HER2 status showed a direct correlation to tumor type and grade of ductal carcinoma. However, a difference existed in the relatively lower ER positivity in patients aged >50 years and the higher percentage of triple-negative cases [35].

HER2/neu and MUC1 receptors: The receptors for HER2/neu and MUC1 are overexpressed in various breast and ovarian cancers. The comparatively low expression of these antigens on normal tissues makes them attractive targets for tumor imaging. Additionally, antitumor-antibody-derived peptides based on the Glu-Pro-Pro-Thr (EPPT) sequence are prepared for the detection of breast cancer. The peptides exhibited good stability *in vitro* in human plasma and against cysteine and histidine challenge and displayed high affinities for MCF-7, MDA-MB-231 and T47-D breast cancer cell lines *in vitro*. The combination of favorable *in vitro* and *in vivo* characteristics makes this new and interesting class of peptides potential candidates for the diagnosis of breast cancer *in vivo* [36].

Epidermal Growth Factor Receptor (EGF-R): Epidermal growth factor receptor and its ligand transforming growth factor-alpha (TGF-alpha), when activated by autocrine growth factors play an important role in BC. The EGFR promotes proliferation, migration, invasion and cell survival as well as inhibition of apoptosis and has been linked to a poor prognosis in BC. Detection of the co-expression of EGF-R and TGF-alpha is an independent prognostic indicator of BC [37-39].

Genes

HER-2/Neu: Tulbah *et al*. [40] evaluated the efficiency of HER-2/Neu overexpression in 54 patients of locally advanced BC treated with primary chemotherapy. The response to neoadjuvant chemotherapy and survival were examined against HER-2/neu overexpression as determined by an immunohistochemistry method. The results showed none of the clinical variables were significantly associated with HER-2/neu expression. The authors concluded that HER-2/neu overexpression determined using Hercep Test assay failed to demonstrate a predictive or prognostic role.

Al-Ahwal [41] reviewed the HER-2 status and its correlation with other prognostic histopathological features in a total of 260 patients during 2000 to 2004. Immunohistochemistry of the histopathological specimens showed HER-2/neu in 145 patients out of 260. Among the 145 patients, HER-2/neu over expression was positive in 28.3% and negative in 71.7% patients. There were no correlations observed between HER-2/neu over expression and age, race, histopathology, tumor size, number of positive lymph nodes, tumor grade, lymphovascular invasion, progesterone receptor status. The c-erbB2 gene (HER-2/neu) is expressed in 10-34% of BCs and its expression is associated with poor clinical outcome [33].

p33 (ING1b): The inhibitor of growth gene 1 (ING1) is a modulator of cell cycle checkpoints, apoptosis and cellular aging. The expression of p33 (ING1b) is the most widely expressed ING1 isoform, which can modulate p53, a molecule that is frequently altered in BC. Reduction of ING1 mRNA expression is generally observed in primary BC expressing wild type p53. Nouman *et al*. [42] reported that the function of p53 is dependent on p33 (ING1b), so that inhibition of nuclear p33 (ING1b) expression would be predicted to resolve p53 function. The results of this study revealed that p33 (ING1b) changes were linked with more poorly differentiated tumors. Hence p33 (ING1b) expression could be used as a molecular marker of differentiation in invasive BC.

nm23: A strong association has been reported between reduced expression of the nm23 gene and acquirement of metastatic behavior in some tumors including BC. Early during the pregnancy, both human and murine trophoblast cells show *in vitro* invasive properties similar to neoplastic cells [43].

BRCA1 and BRCA2: The major segments of BRCA1 and BRCA2 genes were screened for disease-associated mutations in Arab and Asian women with BC from the KSA. DNA samples from 29 Arab women and 11 Asian women with unilateral BC were investigated for BRCA1 and BRCA2 mutations. The results showed that both the mutations are likely to contribute to the pathogenesis of familial BC in female patients from KSA [44].

IGHG3, CDK6 and RPS9: In a study on 48 differentially expressed genes in tumors, Bin Amer *et al*. [45] showed that 3 differentially-expressed genes IGHG3, CDK6 and RPS9 in tumors were suggested to play a novel role in breast cancer.

Multiple Different Oncogenes: Multiple different oncogenes (HER2, EGFR, MYC, CCND1 and MDM2) have been reported to be amplified in BC which results in their overexpression and also serve as an indicator of genomic instability. Hence the gene amplifications may have great prognostic significance. The prognostic significance of amplifications of the different oncogenes was assessed in 2197 samples. The amplifications recorded for different genes were CCND1 (20.1%), HER2 (17.3%), MDM2 (5.7%), MYC (5.3%) and EGFR (0.8%) of the tumors.. All gene amplifications were significantly associated with high grade. The results of this study indicated major prognostic impact of genomic instability as determined by a broad gene amplification survey in BC, in addition, a gradual decrease of survival with increasing number of amplifications was observed [46].

Genetic Polymorphism: Single-nucleotide polymorphisms (SNPs) are observed in many women. There are reports in the literature of the association of SNPs to genetic predisposition to breast cancer, under the influence of nutritional and environmental factors. Furthermore, the tumor suppressor TP53 and its negative regulator MDM2 play significant roles in carcinogenesis in general. However, Alshatwi *et al*. [47] performed a case-control study of patients with breast cancer and healthy controls in a Saudi population using Taq Man-based real-time PCR. The results showed that polymorphisms of MDM2 and TP53 genes may be a genetic modifier for development of breast cancer in this population of Saudi Arabia.

Certain SNPs in genes like p21 or bcl-2 increase susceptibility to BC, however; it is not known whether the common polymorphic variants in the same genes may also

increase risk in Saudi Arabian population. Alshatwi *et al*. [48] investigated to find whether polymorphisms of p21 or bcl-2 might be associated with an increased risk of BC in Saudi women. The results showed p21 (rs733590) C/T SNP was not associated with BC pathogenesis, while bcl-2 genotypes were marginally associated overall with BC risk. However; the alleles of this gene were significantly associated with risk of BC. The authors suggested that it is likely that these genes might increase risks of BC.

Methylation Events: In an effort to understand the molecular signature of BC in Saudi population, Buhmeida *et al*. [49] undertook an investigation to profile the methylation events in a series of key genes including Ra1GDS/AF-6, RASSF1A, H1C1, CDKN2A, RARB2, ESR1, PGR, PITX2, SFRP1, MYOD1 and SLIT2, using Methy Light analysis in archival tumor samples. The results showed that overall methylation levels were low, with only 84% of cases displaying methylation in one or more of the analyzed genes. The frequency of RASSF1A methylation was highest (65%). The authors concluded the usefulness of RASSF1A methylation status as an informative prognostic biomarker in BC in Saudi population.

Epigenetic Modifications: Al-Moghrabi *et al*. [50] investigated the epigenetic modifications of the breast cancer type 1 susceptibility gene (BRCA1) in breast tissues and blood cells of BC patients. The BRCA1 promoter methylation was examined by methylation-specific PCR. The methylation status of the BRCA1 promoter was confirmed and analyzed at high resolution by sodium bisulfite genomic sequencing. The results indicated a possible implication of BRCA1 promoter methylation in the early onset of BC and propose the use of this epigenetic modification as a powerful molecular marker for detecting women potentially predisposed to cancer.

Micro RNAs: Micro RNAs (miRNAs) are a class of naturally occurring small noncoding RNAs that regulate gene expression, cell growth and apoptosis. They have been recently reported as useful biomarkers in diseases including cancer. In a study on 100 BC patients and 89 healthy patients, Alshatwi *et al*. [47] performed miRNA genotyping and expression profiling study using peripheral blood to detect and identify characteristic patterns. The results demonstrated hs-miR-196a2 and hs-miR-499, hs-miR-146a, hs-miR-196a2 in pre- and post-

menopausal BC patients, respectively. Furthermore, a significant association between two microRNA polymorphisms (hs-miR-196a2 and hs-miR-499) and breast cancer risk was found. The authors concluded that peripheral blood miRNAs and their expression and genotypic profiles can be developed as biomarkers for early diagnosis and prognosis of breast cancer.

Flaws Recorded in Mammography, F-18 FDG Uptake and Cytology: A mammography program conducted in Al-Qassim (Saudi Arabia) on 1628 women, showed the cancer detection rate to be 0.24 per cent out of 1.5 per cent biopsies performed. It is matter of great agony that the patients were exposed to biopsy without proper diagnosis and majority had to undergo the invasive procedure. Furthermore, in the same study, it is found that most of the performed indicators were not available and many of the available indicators did not meet international standards [11].

Bakheet *et al*. [51] reported that acute or chronic infectious mastitis and postsurgical hemorrhagic inflammatory mastitis should be considered in patients who have a breast mass, especially those with a history of tenderness or surgery. Although, the whole body Fluorine-18 (F-18 FDG) PET scanning has been a useful technique in the management of BC, but the F-18 FDG uptake has been linked with benign breast disease. Four cases are reported of F-18 FDG breast uptake caused by infectious or inflammatory mastitis that mimics malignant disease.

In a study on cytohistological discrepancies and misinterpretations analyzed on fine needle aspiration cytology material, Jamal and Mansoor [52] reported that hypocellularity and nuclear monomorphism are the reasons for failure to diagnose malignancy in BC. Overcrowded clusters and hypercellular smears needs careful analysis for uniformity of cells and detailed nuclear and cytomorphological features. F-18 FDGif full-blown malignant cytomorphological changes are not detected, a suspicious or inconclusive diagnosis should be made.

Advances in BC Researches in the KSA Sought: Survival and recurrence rates in BC are inconstant for common diagnoses and hence, the biological underpinnings of the disease that determine these outcomes are yet to be fully inferred. With advancements in genetic and imaging techniques, archived biopsies can be examined for purposes other than diagnosis.

A comparison of researches of our findings in the KSA with a report in the literature [53], genes involved in regulation of transcription, oncogenesis, suppression of immune response and drug resistance and recurrence of cancer are yet to be investigated thoroughly. Abramson *et al*. [54] reported new strategies for the treatment of BC which focused extensive target identification and understanding the expression, regulation and function of critical signaling pathways involved in BC initiation and progression. Literature published from our laboratories is deficit of such findings which are the bases for significant increases in median survival for patients with HER2-overexpressing BC. We are yet to define effective agents that can treat HER2-overexpressing BCs, while minimizing toxicity. Studies to address lengthy duration of therapy, the superiority and side-effect profile of different biological drug combinations and determination of biomarkers of resistance to HER2 therapy would be instrumental in decreasing morbidity and mortality for patients with HER2-overexpressing breast cancer.

Although, most of the technological and scientific innovations including mass spectrometry, high-throughput ELISA, tissue or protein microarray techniques [55] have been adopted in the BC researches in the KSA, some techniques such as stable isotope labeling with amino acids and click chemistry are yet to be realized in the management of BC research.

Literature reports suggest that the process of Epithelial to mesenchymal transition (EMT) is associated with the most aggressive type of BC, including the triple-negative breast cancer (TNBC). Kong *et al*. [56] showed that expression of NEDD9 was frequently upregulated in both the TNBC cell lines and in aggressive breast tumors. Reduction of endogenous NEDD9 inhibits the migration, invasion and proliferation of TNBC cells. The authors revealed that NEDD9 promotes EMT and provide useful clues to the evaluation of NEDD9 as responsive molecular target for TNBC and aggressive cancer, chemotherapy. Saudi scientists are yet to explore such innovative experiments to see the effect of some novel compounds on EMT.

Notwithstanding the advances in adjuvant endocrine treatment for hormone receptor-positive tumors and with trastuzumab for HER2-positive disease, over 50% of women with early-stage BC still experience recurrence and die of the disease. Biomarkers for tailoring systemic adjuvant treatment are needed. The multigene assays, 21-gene recurrence score (Oncotype DX [Genomic Health, CA, USA]) and 70-gene signature (Mamma Print [Agendia, CA, USA]) and the isolated tumor cells in sentinel lymph node(s) represent the recent advances to improve adjuvant chemotherapy decisions [57]. These new markers added to standard factors (age, tumor size, grade, hormone receptor status and HER2 status), can improve early BC treatment decisions. BC researches in the KSA are required to adopt the strategy.

Circulating tumor cells CTCs are epithelial tumor cells detected in the peripheral blood of patients with solid tumors. The circulating nucleic acids, microRNAs and genomic rearrangements have been suggested as promising blood biomarkers [58]. However, currently, there is no role for CTCs in clinical practice. The clinical utility of CTCs and other blood biomarkers should be prospectively tested. Ignatiadis *et al*. [59] reported that circulating tumor cells might become a valuable tool to refine prognosis in early and metastatic breast cancer. Circulating tumor cell phenotyping/profiling may serve as a real-time tumor biopsy for individually-tailored targeted therapies. Routine monitoring of CTCs has been advocated as a unique means of detecting BC progression earlier and identifying alterations in tumor cells that might herald the need for changes in therapy. Ongoing researches might help to show the significance of the use of metabolomics and CTC evaluation as new strategies to monitor cancer progression and identify markers of chemotherapy activity and toxicity [60].

CONCLUSION

Breast cancer affects women of all socioeconomic levels in both developed and developing countries. Since BC is more easily treated, if it is detected early, considerable global efforts have been focused in this direction and the KSA is not lagging behind. This review has targeted different procedures commonly used in the early diagnosis of BC in the KSA. These include mammography, ultrasound, biopsy (fine needle aspiration, surgical biopsy), computerized tomography, positron emission tomography (PET), histopathology, cytology and sentinel lymph node biopsy. These methods are the bases for advanced research on molecular markers. In addition to the routine methods employed in the hospitals and research centers, there has been substantial research on biomarkers used in the detection of BC. These include; proteins, nucleosides, hormone receptors, Epidermal growth factor receptors, genes including; HER-2/Neu, p33 (ING1b), Nm23, BRCA1 and BRCA2, IGHG3, CDK6 and RPS9. Additionally, there has been considerable

research on multiple different oncogenes, genetic polymorphism, methylation events, epigenetic modifications and Micro RNAs. The review has also recorded flaws in some methods. Although there has been considerable improvement in researches on biomarkers, there is still a lot to be done in comparison with global achievements.

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