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Nibrin and IL20 Potential Association With Breast Cancer: At Both Benign and Malignant Levels

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Abstract

Breast cancer is a major cause of malignancies–related mortalities among women worldwide. The development and progression of this disease are attributed to the contribution of key cellular modulators, including the *NBN* (Nibrin) gene and interleukin 20 (IL20), that are linked to genomic instability, stemness, cell cycle regulation and cancer predisposition. Due to the lack of local studies investigating *NBN* gene expression in the context of breast cancer, the present study aims to assess its gene expression along with the assessment of interleukin 20 (IL20) serum levels in newly diagnosed breast cancer patients in comparison to cases with benign breast lumps and healthy controls. Total RNA was extracted from the collected blood samples of all 118 participating subjects, including 73 with breast tumor (50 malignant breast tumor and 23 benign breast tumor patients) and 45 healthy controls. Relative *NBN* gene expression was estimated using q-PCR, while IL20 serum levels were assessed using the ELISA technique. Additionally, a number of socio-demographic/clinical features (age, BMI, breastfeeding, menopausal status and family history) were evaluated for associations between them and the measured parameters. *NBN* gene expression levels were significantly upregulated ($p \leq 0.05$) in both malignant breast cancer group (2.33 ± 0.41) and benign breast lumps (1.25 ± 0.25) compared to that of the healthy control group. Additionally, IL-20 serum levels showed significant differences ($p=0.031$) between benign and malignant breast cancer patients (0.69 ± 0.014 and 0.64 ± 0.009, respectively) in comparison to healthy controls. This finding suggests the potential involvement of IL-20, as a stemness modifier in breast carcinogenic events. Overall, the present study findings support the association of *NBN* and IL20 overexpression to breast cancer pathogenesis, with the potential to be involved in precancerous events via its contribution to breast lumps development.

Keywords: Breast cancer, *NBN* expression, IL 20, benign breast lumps, Malignant.

أمكانية ربط النايبرين والانترلوكين 20 مع سرطان الثدي: على كلا المستويين الحميد والخبيث

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الخلاصة

يُعَدُّ سرطان الثدي أحد الأسباب الرئيسية للوفيات المرتبطة بالأورام بين النساء في جميع أنحاء العالم. ويُعزى تطور هذا المرض وتقدمه إلى مساهمة بعض المنظمات الخلوية الرئيسية، بما في ذلك جين (*NBN* (Nibrinوالانترلوكين 20 (IL20) ، اللذَين يرتبطان بعدم استقرار الجينوم، والخلايا الجذعية، وتنظيم دورة الخلية، وقابلية الإصابة بالسرطان. ونظرًا لندرة الدراسات المحلية التي تبحث في التعبير الجيني للـ NBN في سياق سرطان الثدي، تهدف الدراسة الحالية إلى تقييم تعبيره الجيني إلى جانب تقييم مستويات IL20 في مصل الدم لدى مرضى سرطان الثدي الذين تم تشخيصهم حديثا بالمرض وذلك بالمقارنة مع حالات كتل الثدي الحميدة ومع الأفراد الأصحاء من النساء. تم استخلاص الحمض النووي الريبي (RNA) من عينات الدم التي تم جمعها من 118 نساء مصابات بأورام الثدي [تضمنت 73 مريضًا يعانون من أورام الثدي (من بينهم 50 حالة مصابة بسرطان الثدي الخبيث و23 حالة مصابة بأورام حميدة في الثدي) بالإضافة إلى 45 شخصًا من الأصحاء كمجموعة ضابطة]. تم تقدير التعبير النسبي لجين NBN باستخدام تقنية q–PCR ، بينما تم قياس مستويات IL20 في مصل الدم باستخدام تقنية ELISA .بالإضافة إلى ذلك، تم تقييم عدد من السمات الاجتماعية والديموغرافية–/والسريرية (العمر ، ومؤشر كتلة الجسم، والرضاعة الطبيعية، وحالة انقطاع الطمث والتاريخ العائلي للسرطان) للارتباط بينها وبين المعلمات المقاسة. أظهرت النتائج أن مستويات التعبير الجيني NBN كانت مرتفعة معنوياً ($p \leq 0.05$)في كل من مجموعة سرطان الثدي الخبيث (2.33 ± 0.41) ومجموعة الأورام الحميدة (1.25 ± 0.25) مقارنة بمجموعة الأصحاء . بالإضافة إلى ذلك، أظهرت مستويات IL20في مصل الدم فروقًا معنوية ($p = 0.031$) بين مرضى سرطان الثدي الحميد والخبيث (0.69 ± 0.009) , (0.64 ± 0.014 بالتتابع عند مقارنتهم بالأفراد الأصحاء. تشير هذه النتائج إلى الدور المحتمل لإنترلوكين 20–IL كمُحور للخلايا الجذعية يساهم في الأحداث السرطانية المرتبطة بسرطان الثدي. وبشكل عام، تدعم نتائج الدراسة الحالية ارتباط الإفراط في التعبير عن *NBN* وIL20 بتطور سرطان الثدي، مع إمكانية المشاركة في المراحل الأولية للتسرطن من من خلال مساهمته في تطور كتل الثدي.

Introduction

Breast cancer (BC) is the most common malignancy diagnosed in women worldwide and is associated with lower quality of life and increased mortality[1, 2]. As a heterogeneous disease, breast cancer is mainly categorized by a large spectrum of genetic alterations that have the potential to influence its progression (such as HER2 and ER) [3]. The cancer statistics have revealed that BC represents approximately one-third of females' malignancies. About 276,480 new cases were expected, and more than 42,000 estimated deaths cases [4]. A number of molecular biomarkers have been identified to retain different diagnostic, prognostic, and predictive prospectives that impact the disease's initiation, progression and patient stratifications [5].

A part of the diagnostic potential of *BRCA1* and *BRCA2* genes is related to a considerable number of hereditary breast cancer. The efforts have failed to identify additional high-risk related genes that could interpret BC clumping in non *BRCA1/2* families. Though, different moderate or low-risk-stratification genetic abnormalities have been suggested, where the majority of them share similar DNA repair function to *BRCA* genes [6]. Studies have highlighted a significant association between insufficient DNA repair and breast tumor susceptibility [7]. Indeed, a number of stress factors, including free radicals, ionizing radiation, and other unhealthy environmental/lifestyle factors, have the potential to damage genetic material, triggering DNA double-strand breaks (DSBs). This induced DSBs, if left unprepared, could lead to genomic instability and increase the risk of breast cancer initiation [8]. Consequently, identifying disease-associated biomarkers aids with the diagnosis, prognosis, and prediction of breast cancer could have a crucial role in early detection, patients' stratification, facilitating drug discovery and effective disease management [9, 10].

NBN is a gene mapped to chromosome 8q21 and codes for a protein known as nibrin, (also called Nbs1 or p95). This gene product is thought to be involved in DSBs repair, DNA damage-induced checkpoint activation, and maintenance of telomere integrity[11].
Telomere maintenance is a key process for maintaining genome integrity in both normal and cancer cells. Indeed, functional telomeres are indispensable, and without them, chromosomes lose their protective structure and go through fusion and breakage events that drive further genome instability. Moreover, *NBN* is a key component of MRN complex (MRE11, RAD50, and NBN) genes that have a pivotal role in the maintenance of genomic stability. Consequently, any genetic variation of the *NBN* gene may have detrimental effects on cells capability to properly face DNA damage and, consequently, may predispose to cancer [12]. Several lines of evidence have shown that aberrant *NBN* gene expression may be associated with an increased risk of tumorigenesis. Additionally, studies have highlighted that *NBN* polymorphic alterations and any defective mutations occurring in this gene increase the possibility of breast cancer through the dysregulation of the DSB repair mechanism [13]. *NBN* genetic variations were also present in high-risk breast cancer cases [14]. Uzunoglu and colleagues have reported that the interaction between *NBN* and breast cancer 1 gene (*BRCA1*), early onset is facilitated by the 8360 G>C variant at the coding region of *NBN* gene at the C-terminal domain of the protein. This interaction eases the construction of the *BRCA1*-associated genome surveillance complex, which has a role in the recognition and repair of damaged DNA [15]. A large-scale study conducted by the Mavaddat research team (4,500 breast cancer cases and controls) has identified a significant association ($P<0.0065$) between the 30537 G>C *NBN* gene variant and breast cancer[16].

Evidence explaining why the breast is the most organs in the woman's body affected by cancer. It relies on the stem cells reservoir resides, e.g. mammary stem cells, in this organ. Considering that self-renewal is the striking parallel between cancer and stem cells, aberrant stem cell modifiers by cytokine such as interleukin 20 (IL20). IL20 seems to contribute to increasing breast cancer susceptibility. Indeed, IL20 plays a critical role in a number of diverse mechanisms facilitating stemness. For example, the expression of interleukin 20 receptor alpha IL20RA and IL22RA1 promotes stemness in breast cancer [7] and pancreatic cancer [8]. Additionally, IL-22 has been confirmed to boost stemness in colorectal cancer [9] and KRAS-mutant lung cancer [10]. Poor clinical outcome breast cancer patients are shown to exhibit elevated expression levels of IL20. However, genomic instability is an essential driver for cancer development. The accumulation of mutation by this instability and DNA damage is believed to contribute to aging by promoting senescence, cell death, and tissue dysfunction. However, emerging evidence shows that inflammation response is another major significant contributor to DNA damage associated with tumorigenesis [17].

Regardless a considerable amount of literature has been published on the influence of *NBN*. However, no previous studies have addressed the impact of *NBN* expression in breast cancer locally. Thus, the present study was set to evaluate the expression of the *NBN* gene in Iraqi women with breast cancer in comparison to those with benign breast lumps and healthy controls, especially in the context of the association with the stemness modulator interleukin (IL20).

Materials and methods

Ethical Approval

The study design has been approved by the College of Science Research Ethics Committee at the University of Baghdad (Ref. No.0923/0074, dated to the 25th of September 2023). Written consents were obtained from all participants to participate in the present study.

Subjects and Samples Collection

Estimation of NBN expression level by qRT-PCR

Blood samples were collected from a total number of 118 female participants with breast tumors and healthy controls (age range 18 to 70 years) during the period extended from December 2023 to June 2024 for molecular analysis. Samples and clinical data were obtained from the histopathology reports of Al-Yarmouk Teaching Hospital, Al- Amal Hospital, and Al-Alwiyya Maternity Teaching Hospital in Baghdad- Iraq. All patients were diagnosed according to the adopted hospitals clinical protocols through ultrasound and mammography examinations. Participants were subdivided into three groups: Group 1 was assigned as the control group consisting of 45 healthy females, Group 2, included 23 patients with benign breast lumps, and Group 3 included 50 females with newly diagnosed malignant breast cancer. A number of demographic characteristics (age, weight, and height) were also collected from all participants (breast cancer, benign breast lumps, and healthy controls).

Estimation of NBN expression level by qRT-PCR

For RNA extraction, a volume of 250 µl of the collected blood samples from each participant was added to 500 µl of TRIzol® LS reagent (Invitrogen Company, USA) in Eppendorf tubes. The RNA was extracted using Easy Pure® Blood RNA Kit (TransGen Biotech Company, China) according to the protocol provided by the manufacturer. The extracted RNA was then converted to cDNA by ProtoScript® First Strand cDNA Synthesis Kit. This procedure included adding 10µl Protoscript reaction mix for each sample, then 2 µl per sample from MuLV Enzyme, then added 2 µl of oligoT and 3 µl from each extracted total RNA sample, all added into a new PCR tube, and the volume completed up to 20 µl by adding nuclease-free water (NFW). This mixture was incubated for 1 hour at 42 ºC using a PCR thermocycler, and this was followed by 80 ºC for the inactivation of the enzyme. The component of the qPCR protocol for gene expression determination was Luna universal qPCR master mix10 µL and 0.5 µL for each forward and reverse primer, 4µL from template DNA, and completed the volume to 20µL with NFW. *GAPDH* as a housekeeping gene was used in this study. The primers sequence used for the assessment of NBN relative expression are as following: *NBN* forward 5′-GTTGATCTGTCAGGACGGCAG -3′, *NBN* reverse 5′-TCCCCACCTCCAAAGACAACT -3′. *GAPDH* primer sets were adapted as a housekeeping gene with the forward primer sequence 5′-GTCTCCTCTGACTTCAA-3′, and *GAPDH* reverse primer sequence 5′-ACCACCCTGTTGCTGTA-3′. The quantitative real-time qRT-PCR analysis method was used to assess *NBN* gene expression in the investigated subjects.

NBN expression fold change was estimated through the following equations:

ΔCt (control) = CT (*NBN*) – Ct (*GAPDH*).

ΔCt (patient) = CT (*NBN*) – Ct (*GAPDH*).

ΔΔCt = ΔCt (patient) - ΔCt (control)

Ultimately, fold change of the *NBN* relative gene was calculated *via* the "$2^{-\Delta\Delta Ct}$" Livak's equation [18, 19].

Assessment of IL20 serum levels by ELISA technique

The serum level of IL20 was assessed in all of the investigated participants. For this purpose, serum was separated from the collected blood samples by centrifugation at 5000 rpm for 5- 10 min at room temperature, and the serum was stored in an Eppendorf collection tube at -20 ºC until use. The venous blood samples (5 ml) were collected from each participant. IL 20 ELISA kit (Cat. No. ELK1291/ELK Biotechnology) was used according to the manufacturer's instructions. After the preparation of all solutions as in the kit procedure, the concentrations of standards were as follows: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25

pg/mL, and 15.63 pg/mL respectively, the last Eppendorf tube with standard diluent is the blank as 0 pg/mL. Determined the first wells in IL 20 ELISA Kit for diluted standard, blank, controls, and then samples (malignant and benign). Then, the microplate reader ELISA system (RT-2100C Microplate Reader, Germany) was used to measure the OD at 450 nm as the manufacturer's kit. The optical density for each standard, control, and samples were calculated. The standard curve for the Human IL20 was determined as standard concentration on the Y-axis and absorbance on the X-axis.

Statistical Analysis

All data were analyzed using SPSS (version 27, IBM), GraphPad Prism (version 8.4), and Microsoft Excel 2016. Statistical analyzes included analysis of variance One-Way ANOVA variance analysis to analyze the difference between the means of more than two groups (as age categories) and independent t-tests, as appropriate, to determine p-values were adopted from the assessed parameters' mean comparisons (menopausal status, contraceptive and breastfeeding data). Data are presented as Mean ± S.E., with a p-value of <0.05 considered statistically significant [20].

Results

The diagnostic breast cancer patients aged 61 years and older were shown to have significantly (P=0.032) higher *NBN* gene fold expression (4.15 ± 1.8). However, the serum levels of IL-20 did not show significant differences among the different assessed age groups (Table 1), suggesting increased levels regardless of the breast cancer disease presentation.

Table1: *NBN* gene expression fold and IL20 serum level in the studied breast cancer patients based on their age at diagnosis

		Parameters (Mean ± SE)	
Age categories	**No**	***NBN* expression fold**	**Il-20 serum level (pg/mL)**
30-40	8	2.4 ± 0.22	0.66 ± 0.03
41-60	56	2.05 ± 0.33	0.65 ± 0.012
61-70	9	4.15 ± 1.8	0.62 ± 0.014
Eta-squared	-	0.086	0.022
P-value	-	0.032*	0.586 NS

- Data are presented as mean ± standard error (P<0.05**), significant or (P>0.05), NS=Non-significant
- Eta squared= One-way *Anova* effect size (Small=0.01, medium=0.06, large=0.14)

With respect to the impact of menopausal status, post-menopausal diagnostic breast cancer patients showed to have significantly (P=0.046) higher *NBN* gene fold expression (2.85 ± 0.48). However, this is being not the case with respect to the IL-20 serum when its levels were comparable in both premenopausal and post-menopausal diagnostic breast cancer patients (Table 2).

Table 2: *NBN* gene expression fold and IL20 serum level in the studied breast cancer patients according to their patients' menopausal status

		Parameters (Mean ± SE)	
Menopausal status	**No**	***NBN* expression fold**	**IL-20 serum level (pg/mL)**
Premenopausal	32	1.51 ± 0.35	0.65 ± 0.02
Post-menopausal	41	2.85 ± 0.48	0.64 ± 0.01
Cohen's d	-	-0.546	-0.012
P-value	-	0.046*	0.809 NS

- Data are presented as mean ± standard error (P<0.05**), significant or (P>0.05), NS=Non-significant
- Cohen`s d=independent t-test effect size (Small=0.2, medium=0.5, large=0.08)

Regarding the association between the investigated parameters (*NBN* genes expression fold and IL-20 serum level) and the clinical feature of the diagnostic breast cancer patients, breastfeeder women showed to have significantly (P= 0.048) higher *NBN* expression fold than those who are not (2.9 ± 0.52 vs. 1.6 ± 0.94, respectively).While *NBN* gene expression fold showed to be relatively lower in breast cancer patients who reported contraceptive use than in non-users, these differences did not reach a significant level (P≥0.05, 1.8 ± 0.46 vs. 2.5 ± 0.42, respectively, Table 3). IL-20 serum levels showed no significant differences according to the breastfeeding and contraceptive use status of the studied breast cancer patients (Table 3).

Table 3: *NBN* gene expression fold and IL20 serum level in the studied breast cancer patients based on their breastfeeding and contraceptive use status

		Parameters (Mean ± SE)	
Breastfeeding	**No**	***NBN* expression fold**	**IL-20 serum level (pg/mL)**
Yes	38	2.9 ± 0.52	0.65 ± 0.01
No	35	1.6 ± 0.94	0.63 ± 0.016
Cohen's d	-	0.565	0.07
P-value	-	0.048*	0.5 NS
Contraceptive use			
Yes	33	1.8 ± 0.46	0.64 ± 0.022
No	40	2.5 ± 0.42	0.65 ± 0.009
Cohen's d	-	-0.302	-0.074
P-value	-	0.267 NS	0.837 NS

- Data are presented as mean ± standard error (P<0.05**), significant or (P>0.05), NS=Non-significant
- Cohen`s d=independent t-test effect size (Small=0.2, medium=0.5, large=0.08)

Notably, *NBN* gene expression folds were relatively similar in all of the assessed body mass index (BMI) categories (normal weight, overweight, and obese: 2.5 ± 0.6, 2.1 ± 0.57, 2.3 ± 0.45, respectively, Table 4) of the studied breast cancer patients. Similarly, IL-20 serum levels were almost the same in the in assessed BMI subgroups of the investigated breast cancer newly diagnosed cases (Table 4).

Table 4: *NBN* gene expression fold and IL20 serum level in the studied breast cancer patients based on their BMI categories

		Parameters (Mean ± SE)	
BMI (kg/m2)	**No**	***NBN* expression fold**	**IL-20 serum level (pg/mL)**
Normal	7	2.5 ± 0.6	0.65 ± 0.03
Overweight	23	2.1 ± 0.57	0.63 ± 0.02
Obese	43	2.3 ± 0.45	0.64 ± 0.012
Eta-squared	-	0.002	0.01
P-value	-	0.949 NS	0.788 NS

- Data are presented as mean ± standard error (P<0.05**), significant or (P>0.05), NS=Non-significant
- Eta squared= One-way *Anova* effect size (Small=0.01, medium=0.06, large=0.14)

NBN gene expression levels were also compared between patients with different breast cancer groups (e.g. begin *vs.* malignant), as shown in Table 5. Patients with benign breast lumps showed to have significantly (P= 0.0007) lower *NBN* gene expression fold than those with malignant breast cancer (Figure 1). This finding suggests that an increase in *NBN* gene expression fold could be associated with the increased breast carcinogenicity level.

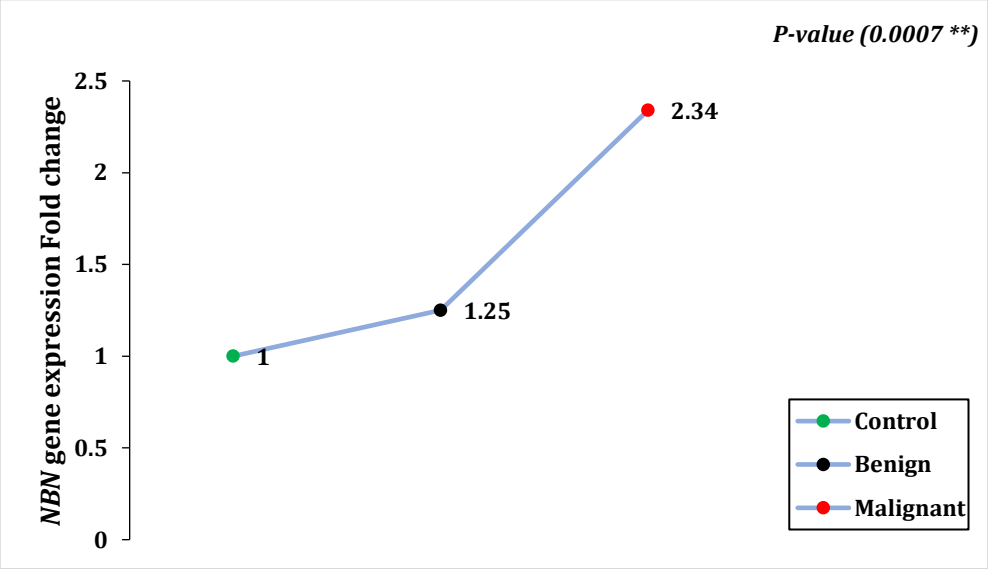

Figure 1: *NBN* gene expression folds in patients with benign breast lumps and malignant breast cancer in comparison to healthy controls

However, IL-20 serum levels showed significant differences (P=0.031) between benign and malignant breast cancer patients in comparison to healthy controls (Figure 2). This finding suggests the potential involvement of IL-20 as a stemness modifier that contributes to breast carcinogenic events.

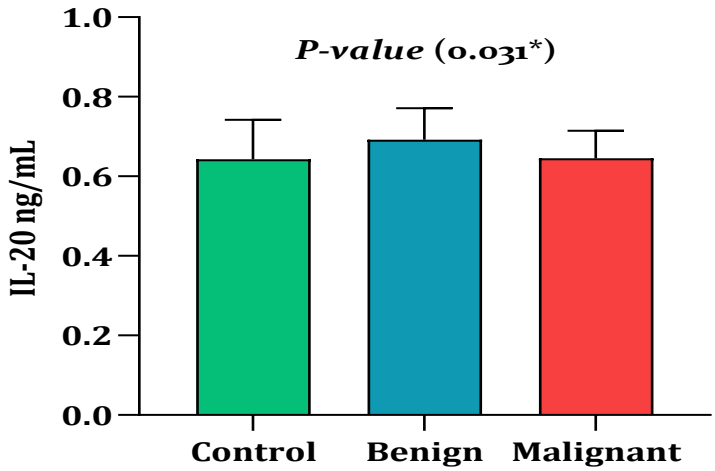

Figure 2: IL-20 serum levels in patients with benign breast lumps and malignant breast cancer in comparison to healthy controls

On the other hand, IL-20 serum expression levels showed more elevated in benign lumps compared to malignant breast cancer patients (0.69 ± 0.014, 0.64 ± 0.009), respectively, as appeared in Table 5.

Table 5: *NBN* gene expression fold and IL-20 serum expression level in different breast cancer study groups

		Parameters (Mean ± SE)	
Groups	**No.**	***NBN* expression level**	**IL-20 serum level (pg/mL)**
Control	45	1 ± 0	0.64 ± 0.03
Benign	23	1.25 ± 0.25	0.69 ± 0.014
Malignant	50	2.33 ± 0.41	0.64 ± 0.009
P-value		0.062*	0.031*

Discussion

Identifying the differential expression level of a number of genes, key cellular components, and pathways could have a crucial role in understanding the underlying biological events associated with cancer development [21-24]. Additionally, studying cytological and molecular events that are related to breast cancer development, the most frequent cancer among women worldwide, may aid with the development of prevention and therapeutic strategies to challenge such destructive health issue [25-27]. It is well acknowledged that the loss of function of DNA damage repair (DDR) genes, e.g. *P53* gene that is associated with up to 50% of different types of known malignancies, has a significant impact on the lack of maintaining genome integrity that contributes to tumorigeneses [28]. However, overexpression or the gain of more functions of such genes has also been associated with cellular transformation and drug resistance of cancer cells. Such a contrasted effect may play a detrimental role in cancer development [29]. In the present study, the expression of the *NBN* gene as a breast cancer-related gene, which is an integral part of the genome repair system, is highly elevated in the studied breast cancer patients. Actually, *NBN* gene expression was much higher in patients with malignant breast cancer than that of benign breast lumps and healthy controls, implying the potential need for maintaining increased *NBN* gene expression level to meet the need of cancer cells to continue their uncontrolled proliferation. This notion has been further supported by the finding of Belhadj and colleagues' recent study that reported the role of *NBN* gain of function genetic alteration is related to drug resistance and increased survival of different types of cancers cells, including breast and ovarian cancer. Thus, understood the fact that tumors retain *DDR* gene

expression augmentation (e.g. *NBN*). Exhibited lower rate of mutation spectrum and mechanism-specific mutation malignant phenotype, imposed an enlarged DDR efficiency in the DDR amplified tumors. Clinically, the outcome of patients with *DDR* gene amplification is extremely poor, as it is shown by different types of cancer. Also, *NBN* is reported to be subjected to different types of genetic alterations, including amplifications and SNP variations that are linked to different types of cancer, including prostate, lymphoid, and colorectal malignancies[12].

NBN gene expression was showen to increase in all breast cancer patients in both younger ages (<40 yrs) and those aged older (>60year), suggesting a general influence of its expression over the different age groups. This is quite an interesting observation and needs to be considered in a larger cohort of younger age breast cancer patients to validate its clinical significance. Also, *NBN* expression fold changes showed to be almost doubled in post-menopausal women with breast cancer. Although *NBN* is overexpressed in all of the studied breast cancer patients' groups, its expression was relatively higher in breast cancer with older age as well as in those with normal weight and obese breast cancer patients. This suggests a general crucial role for *NBN* expression in breast cancer pathogenicity. Accordingly, the overexpression of *NBN* could provide actionable steps for the development of clinical biomarkers for cancer management [30].

Globally, the occurrence of breast cancer among young women (BCYW) has been markedly increasing [31,32]. Furthermore, increased BMI is also blamed for breast carcinogenesis. This is evident as both malignant breast cancer patients and cases with benign breast lumps showed to have significantly elevated BMI levels. Considering the fact that cancer is an age-related disease, BCYW remains poorly understood, implication the need to fill this knowledge gap with respect to the disease risk stratification [31, 33]. *NBN* gene expression showed an increase in both pre and post-menopausal breast cancer patients with higher expression fold change associated with the last group. This finding suggests a general trend to increase its levels regardless patients' menopausal status with potential influence in inducing breast tumorigenesis. In our opinion, it is associated with breast remodeling; additionally, cellular and genetic alteration is related to women in this state. Furthermore, women who used to breastfeed their babies and were affected with breast cancer showed to have relatively higher *NBN* expression than those who were not. This may suggest a positive impact of breastfeeding on *NBN* expression in the context of breast carcinogenesis. However, the use of contraceptive pills seemed to do the opposite in terms of the association with *NBN* expression fold change, where non-user breast cancer patients showed to have relatively higher expression levels of *NBN*. Thus, breastfeeding is believed to be the most significant factor influencing breast cancer development, and it is thought that breast cancer risk is decreased by 4.3% for every twelve months of breastfeeding [34].

The present study also examined the potential association between IL20 serum levels and breast cancer where its average levels showed to be significantly increased in patients with benign breast lump over malignant breast cancer cases and healthy controls. Regardless of the acknowledged association between inflammation and tumorigenesis[35]. IL20 could play a crucial role in enhancing stemness and promoting the creation of an immunosuppressive microenvironment in breast cancer [36]. Additionally, previous studies have linked IL20 to promote cancer growth and metastasis. Chemotherapy resistance and poor outcomes in breast cancer patients have also been shown to be associated with elevated IL20 levels [37].

One limitation of the present study is that it mainly focused on the assessment of newly diagnosed breast cancer cases where follow-up data were not available, thus, large-scale studies

are recommended to validate the current study findings, especially in relation to well-established breast cancer prognostic biomarkers. This would open a new venue for breast cancer patients' stratification and the development of new therapeutic targets [38].

Conclusion

The present study findings have highlighted the significant association between *NBN* expression alterations, and to a lesser extent with IL20, with breast cancer pathogenesis at both benign and malignant levels. If such findings could be confirmed in larger cohort studies, both *NBN* and IL20 expression levels could be used for breast cancer patients' risk stratification and a biomarker for the development of novel therapeutic strategies.

Confect of interest

The authors declare that they have no conflicts of interest.

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