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ORIGINAL ARTICLE

A DECLINE IN VITAMIN K AND SOD LEVELS AND THE CHANGE IN PARP1 AND SIRT1 EXPRESSION MIGHT BE ASSOCIATED WITH PROGRESSION OF BREAST CANCER

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Summary

Cancer cells have a special energy metabolism that enables them to multiply quickly. Under normal oxygen conditions, the Warburg effect is a distinguishing aspect of cancer metabolism in which anaerobic glycolysis is favored. Enhanced glycolysis also helps to produce nucleotides, amino acids, lipids, and folic acid, all of which are necessary for cancer cell division. In a variety of metabolic processes, including glycolysis, the co-enzyme nicotinamide adenine dinucleotide (NAD) mediates redox reactions. NAD levels that are higher promote glycolysis and supply energy to cancer cells. NAD metabolism, like energy metabolism, is linked in cancer genesis and could be a potential therapeutic target for cancer treatment. In this research, NAD-consuming enzymes, poly(ADP-ribose) polymerase (PARP) and SIRT1, have been investigated in breast cancer patients, in addition to detect the levels of serum malondialdehyde (MDA), superoxide dismutase (SOD) and vitamin K levels. Sixty participants were enrolled in this study, 30 women with breast cancer and 30 controls. Serum were analysed for determination of the levels of PARP1, SIRT1, MDA, SOD, and vitamin K. The results showed a drop in the expression levels of PARP and that was concomitant with the elevation in the expression levels of SIRT1 and MDA, in addition to the drop in SOD and vitamin K levels. These findings suggest that SIRT1 might be the most NAD-consuming enzyme in cancerous cells rather than PARP (the DNA repair enzyme), and this increase in MDA with the drop in SOD and vitamin K might be associated with the increase in cell proliferation and a decrease in apoptotic cell death. Finally, this study could be used as a treatment option for patients with breast cancer. could be used as a treatment option for patients with breast cancer.

Key words: NAD; PARP1; SIRT1; Vitamin K; Breast Cancer Patients

Introduction

NAD as a regulatory step in the Glycolysis, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, and serine production pathways contributing to maintenance of redox status (1). $NADH/NAD^+$ and $NADPH/NADP^+$

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redox levels in the cytosol and mitochondria are intertwined. The relevance of NAD^+ and its redox potential in cellular metabolism is demonstrated by the fact that these states are dependent on cellular mechanisms that create NAD^+ from NADH (2). The delivery for reduced form of NAD is critical for highly multiplying cells to sustain optimal survival and growth, particularly via glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH), both of which require NAD as a co-enzyme (3, 4). In cancer cells, NAD levels are known to be a critical regulator of serine production (5, 6).

NAD functions as a redox coenzyme by dehydrogenases and as a co-substrate by NAD-consuming enzymes in a variety of metabolic intermediate processes (7, 8). Among them are: I mono- or (poly-ADP ribosyl transferases) (including ARTs and PARPS), which ADP trans the moiety ribose to acceptor proteins, resulting in their modification and function regulation; (ii) sirtuins, which catalyze NAD-dependent deacetylation of enzymes metabolic and transcription factors, thereby controlling their activity; and (iii) NAD glycohydrolase (9, 10). (NAMPT, CD38, sirtuins), and IDO are some of the enzymes involved in NAD homeostasis that have shown been to have a role in cancer immunological tolerance (11-13).

On the other hand, numerous NAD biosynthetic routes supply coenzyme in diverse combinations and with varying efficiency, depending on the cell type and metabolic state (14, 15). The NAD^+ pool is maintained through a continuous cycle of biosynthesis and degradation, with enzymatic consumption on one end and the Salvage and Preiss– Handler pathways or de novo production on the other (2) (see Figure 1).

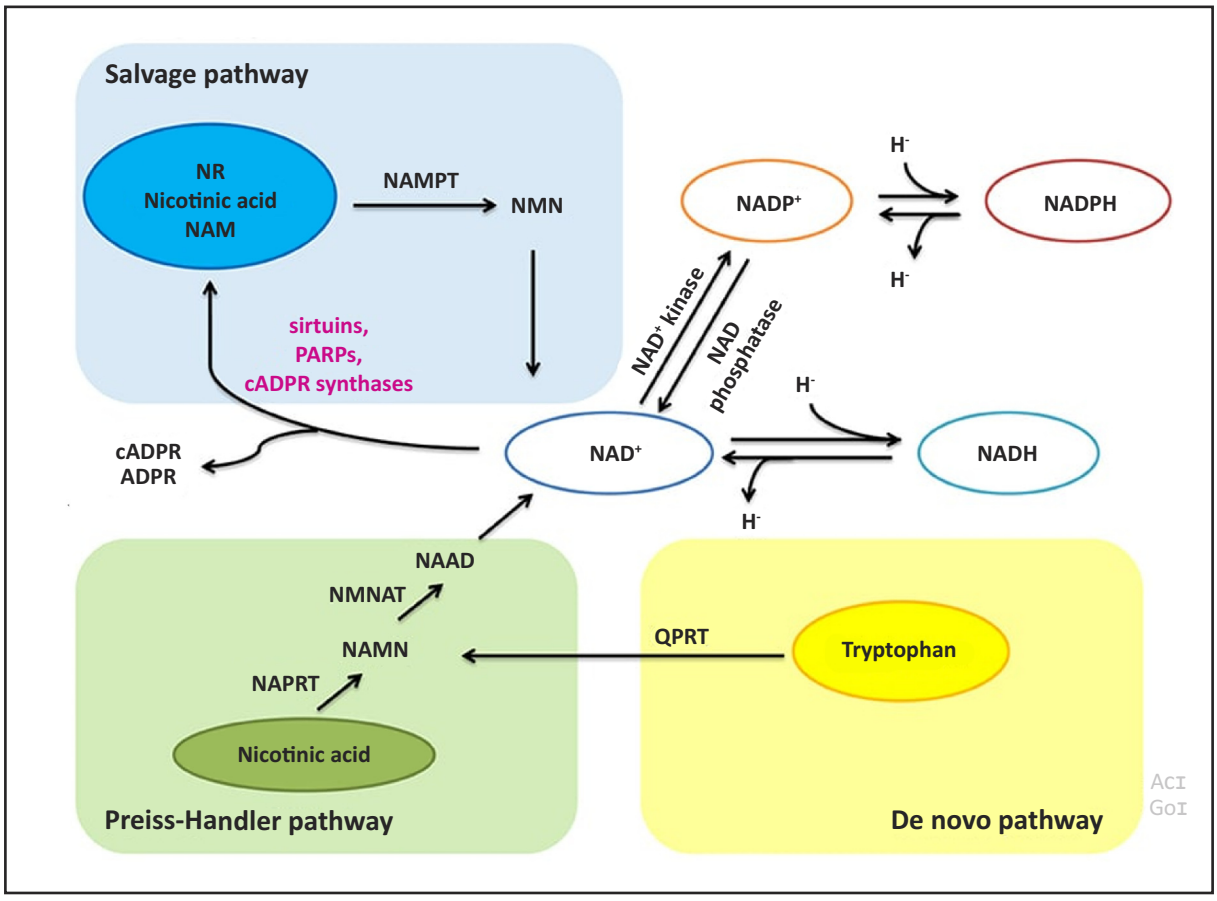

Figure 1. Overall NAD^+ metabolism. Through the *de novo* biosynthesis pathway in the liver and kidneys, NAD^+ can be made from Trp. Nicotinic acid (vitamin B3) enters the NAD^+ pool via the Preiss–Handler pathway, whereas nicotinamide, nicotin amide riboside, and NMN (re-)enter via the salvage pathway. SIRTs, CD38, and PARP enzymes all require NAD^+ [reprinted from Verdin E, 2015 (13)].

Importantly, altered NAD^+ metabolism has been associated to a variety of metabolic and diseases, and therapeutic strategies including modification of the NAD^+ biosynthesis and consumption pathways using (NAD^+ precursors) or pharmacological inhibitors are being researched (16–19). Changes in NAD^+ metabolism, whether through redox metabolism or the activities of (NAD^+consuming) enzymes, have an impact on a variety of cellular processes. It's difficult to say which aspects of metabolism NAD^+ are important for different illnesses, and it's probably a combination of factors. Because PARP-mediated repair DNA requires NAD^+, lowering NAD^+ production may expedite cancer cell death (20). Synthetic lethality was demonstrated in breast cancer cells using a combination of PARP inhibitors and Nicotinamide phosphoribosyl transferase (NAMPT) inhibitors (21). NAMPT and NAD^+ metabolism have been widely studied in metabolic illnesses such as cancer and diabetes in recent studies (22, 23).

Moreover, there have been some discrepancies in the research on the pharmacological modification on vitamin K levels, vitamin K has also been shown to have antiproliferative effects on several types of cancer cells, in addition to the well-known role of PARP inhibitors or SIRT1 inhibitors (24). Several malignancies have been shown to be inhibited by vitamin K2 treatment *in vivo* (25). This sparked a flurry of research on the role of vitamin K intake and supplementation in cancer prevention, progression, and recurrence (26). The vitamin K family consists of fat-soluble compounds with the 2-methyl-1,4-naphthoquinone (3-) groups in common. Vitamin K is available in three different forms: natural K1 and K2 and synthetic K3 or meNADione (27). Interestingly, long-term usage of vitamin K antagonists was linked to a decreased cancer incidence in a meta-analysis of nine observational studies (28). Triple negative breast cancer cells treated to Menaquinone (MK) showed reduced stemness and anti-proliferative effects, which is particularly relevant to breast carcinogenesis (29).

The present study aimed to determine changes in gene expressions of NAD-consuming enzymes (PARP1 and SIRT1), oxidants-antioxidants state and vitamin K levels in breast cancer (BC) patients in order to clarify the mechanism by which vitamin K or NAD metabolic pathways induce their proliferative or anti-proliferative effects which may lead to relevant therapeutic strategies for BC patients.

Materials and Methods

Study Population: The study was conducted in Babylon, city in Iraq. Thirty women diagnosed with breast cancer were selected, during the period from October 2021 to December 2021. Ages ranging from 40 to 60 years, matched for age and sex included in the study.

Patients treatment, characteristics are underwent breast conserving surgery and some patients underwent modified radical mastectomy. All of the patients received postoperative radiotherapy and patients received adjuvant chemotherapy (tamoxifen, xeloda, taxotere and arimidex).

Methods: Each subject's blood (5 mL) was obtained and separated into two tubes (2 ml blood in K2EDTA tube for gene expression was screened by qPCR study and 3 ml in gel tube). The serum was removed from the gel tube of blood by centrifuging it at 3600 rpm for 10-15 minutes at 4 °C and then freezing it for laboratory analysis, such as spectrophotometric MDA values. The immunoassay technique was used to determine the amounts of vitamin K and SOD in the serum (Human vitamin k or Human superoxide dismutase & Elisa Kit, BT-lab China).

RNA was extracted from blood using a TRIzolTM RNA extraction kit and reverse-transcribed using a Proto Script® First Strand cDNA Synthesis Kit and a Luna Universal qPCR Master Mix kit (NEB UK). The PARP or SIRT1-specific forward and reverse universal primers, as well as the cDNA Bright Green master mix, were used to combine the cDNA. As an endogenous control, B-ACTIN was employed. Using the comparative threshold cycle (Ct) and (2 -Ct), the relative levels of PARP or SIRT1 were computed, and the findings indicated the fold change in expression.

Statistical Analysis: GraphPad Prism 9.2.0 and Microsoft Office Excel 2013 were used to summarise, analyse, and show the data. The mean standard error mean was used to express numerical data. In the case of regularly distributed variables, the unpaired t-test was employed to compare mean values between groups. When the p-value was less than 0.05, it was considered significant.

Results

In the present study, in order to see if there was a link between SIRT1 expression and breast cancer progression, the expression of SIRT1 was evaluated in breast cancer patients. The results revealed a higher SIRT1gene expression in BC patients compared to the control group (0.028±0.00304), (4.3330±0.92739) respectively; the significant difference (p value = 0.0024). as shown in figure (2).

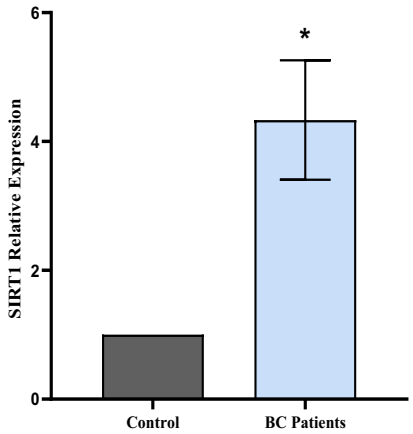

Figure 2. SIRT1 expression patients levels in with breast cancer and control groups, ages average between (35-65) years. Data are expressed as means ± SEM, differences *indicates significant compared to the control, ($P \leq 0.05$).

The change in the expression levels of other NAD-dependent enzymes has been also investigated in patients with breast cancer and were compared to the control . There is a reduction in the expression levels of PARP1 that has been shown, compared to the control group (0.71953 ± 0.12556) ,(26 ± 1.76), (1 ± 0) mg/dL respectively; the significant difference (p-value = 0.0343) as shown in figure (3). Table (1) shows the PARP1 and SIRT1 primers. These results suggest that during cancer the change in NAD+ homoeostasis might affect enzymes levels.

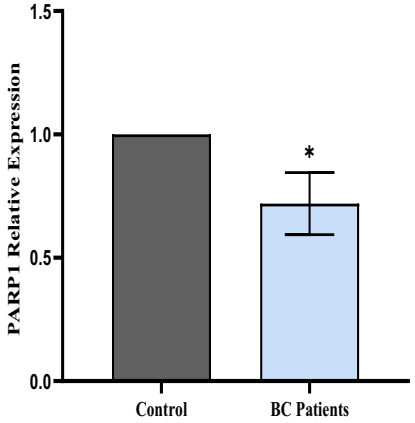

Figure 3. PARP expression patients levels in with breast cancer and control groups, average ages between (35-65) years. Data are expressed as means ± SEM, differences *indicates significant compared to the control, ($P \leq 0.05$).

Interestingly, a clear elevation in MDA levels have been recorded in the patients group compared to the control. There is an increase in the concentration of MDA (mM) in patients as compared with the control group (0.028 ± 0.00304), (0.0049 ± 0.00136) respectively; the significant difference (p-value <0.0001) as shown in figure (4).

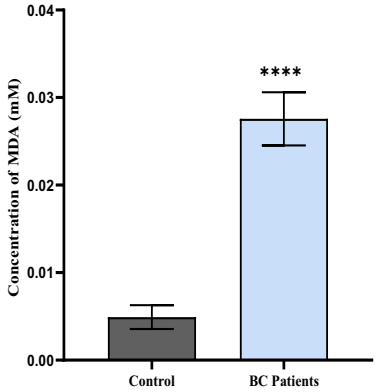

Figure 4. Serum MDA patients levels in with breast cancer and control groups, average ages between (35-65) years. Data are expressed as means ± SEM, differences *indicates significant compared to the control, (P ≤0.05).

The current experiments might reveal a potentially important link between NAD metabolism and oxidative stress, and that endogenous NAD levels determine PARP and SIRT1 proteins synthesis efficacy. In addition to the most interesting and novel link between NAD metabolism and MDA levels.

Table 1. Primers used for qPCR experiments

Genes	Primers	Size	GC%	Product (bp)
B-ACTINE	For: AGGCACCAGGGCGTGAT	17	64.7	51
	Rev: GCCCACATAGGAATCCTTCTGAC	23	52.1	
PARP1	For: GGGATGACCAGCAGAAAGTCA	21	60	108
	Rev: CTGCAAAGTCACCCAGAGTCT	21	59	
SIRT1	For: GCAACAGCATCTTGCCTGATT	21	47	120
	Rev: GATAGCAAGCGGTTCATCAGC	21	52	

Vitamin K and SOD levels were also measured in patients and control groups, a decrease levels in vitamin K (ng/ml) was observed in patients as compared with the control group (344.05± 20.814), (264.99 ± 47.184) respectively; the significant difference (p-value = 0.2992) as shown in figure (5). A similar reduction was also observed in SOD levels in patients compared to the control group (444.09± 2 56.8770.814), (316.21± 45.178) respectively; the significant difference (p-value = 0.1163) as shown in figure (6).

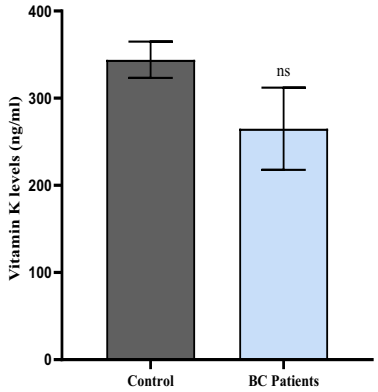

Figure 5. Concentration of vitamin K in serum patients and control group the significant difference (*p-value* = 0.2992). expressed Data are as indicates means ± SEM. *significant differences compared to the control, P≤0.05.

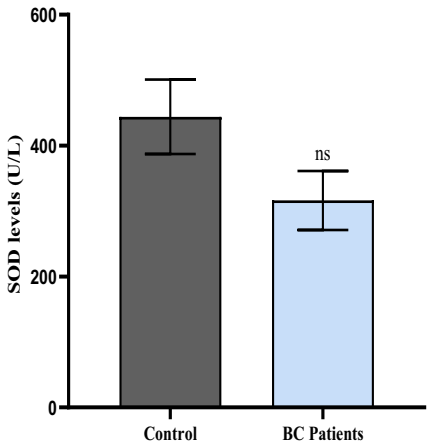

Figure 6. Concentration of SOD in serum patients and control group. Dxpressed Data are as means ± SEM. * indicates significant compared differences to the control, P≤0.05.

Discussion

Breast cancer is one of the most frequent cancers in women around the world. Because there are no accurate predictive biochemical indicators for tumor progression, there are many challenges in predicting tumor progression. As a result, in the current study, we looked at the most essential biochemical markers in order to investigate why breast cancer progresses. Multiple redox pathways energy involving production, glycolysis, the citric acid cycle, oxidative phosphorylation, fatty acid oxidation, and (serine biosynthesis require NAD^+) as a cofactor. NAD^+, on the other hand, is a substrate for a number of signaling enzymes, including sirtuins, PARPs, ARTs, and the CD38/CD157 system (30-32). The benefits of employing NAD^+ boosters and supplementing with NAD^+ precursors to sustain NAD^+ levels in disease circumstances (33). levels NAD^+ have an impact on inflammation, calorie restriction, exercise, DNA repair, lifespan, and health span (34). In a study on a coronavirus-dependent drop in levels NAD^+, the authors suggested using precursors NAD^+ to alleviate the inflammatory state of the lungs (34, 35). Notably, studies on breast cancer ignore the connections NAD^+ between availability and its involvement in the regulation of DNA damage, vitamin K, and cellular metabolism on the one hand, and the expression levels of PARP and Sirtuin (as NAD-consuming enzymes) on the other hand. Currently, it is known that availability NAD^+ influences SIRT1, which has an impact on cellular metabolism via the SIRT1-dependent pathway, but it is possible that when NAD^+ levels are kept high, most NAD^+ dependent enzymes, particularly mono(ADP-ribosyl)ation enzymes, enhance their activity and operate better.

Product SIRT1 was significantly overexpressed in BC patients compared to controls in this study; findings are consistent with those of Hao *et al.*, 2014, who found that SIRT1 was significantly overexpressed in tumour tissues and cell HCC lines, and that significantly SIRT1 promoted the ability of cancer cells to migrate and invade (36). Furthermore, studies using a mouse model demonstrated that overexpression of SIRT1 increased HCC tumour spread *in vivo*. SIRT1 expression was substantially correlated with tumour size, tumour number, and positive SIRT1 expression was associated with poor prognosis in cancer patients, according to a clinical and pathological investigation. SIRT1 is linked to cancer, and its levels differ between organs and tissues. SIRT1 is elevated in cancer colon patients, prostatic carcinoma patients, acute leukaemia myelogenous patients, cutaneous melanoma patients, and non-melanoma skin cancer patients, whereas it is downregulated in BC and hepatocellular carcinoma patients (37). The majority of evidence suggests that SIRT1 can increase tumour growth, although some research suggests that it can also prevent tumour growth. SIRT1 is known to have anti-aging properties, and calorie restriction has been shown to increase its activity, decreasing cancer risk (38,39). SIRT1 is also thought to play a role in cancer cell proliferation, angiogenesis, and metastasis via deacetylating the MAP kinase pathway, which promotes cancer cell proliferation, angiogenesis, and metastasis (41, 40) Our findings show that SIRT1 may play a significant role in the course of BC and could be a potential cancer molecular treatment target. oxidative stress, ion, glycolysis, citric acid cycle.

PARP1, the most well-studied and well-characterized of the members involved in single-strand break repair, is the second key (NAD-consuming enzyme (SSBs). It is triggered when DNA is damaged. Excess PARP1 activation produced by genotoxic substances, on the other hand, can significantly NAD^+ depress and ATP levels, disrupting sirtuin activity and cellular homeostasis, and eventually resulting in cell death (42, 43). It also functions as a tumour promoter or suppressor and regulates cell proliferation, cell cycle, gene transcription, inflammation, and cell fate. PARP1 can be activated in cells by DNADamage and high levels of NAD^+, which is interesting. In the current investigation, however, elevated SIRT1 expression limited the availability of NAD to PARP1, resulting in reduced expression PARP1 Figure (3). Low (PARP-1 expression has also been observed in breast cancer, including BRCA1-associated, triple negative), and basal-like tumours (44), The use of expression PARP1 in tumours to aid in the selection of BC patients for PARP inhibitor therapy could be beneficial.

A substantial increase in MDA levels (Figure 4) could also be a marker of cellular oxidative damage or excessive lipid peroxidation in malignant cells. Free radicals have been linked to the peroxidation of unsaturated fatty acids in breast cancer patients, and obesity has been linked to the development of breast cancer (45). Superoxide radicals are created during this oxidative metabolic process and are eliminated by superoxide dismutase (SOD) enzymes. Despite conflicting results previously published about the tissue and plasma concentration of SOD in patients with breast cancer, low SOD levels were examined (Figure 6) with the accelerated oxidation rate in the current study. This decrease in SOD levels could be related to the progression of the disease (46). Higher oxygen-free-radical generation and lower SOD levels were found in our investigation, which supports the oxidative stress concept in breast carcinogenesis, as a result of DNA damage and excessive levels of free-radicals have already been documented on the tissue and plasma. Finally, vitamin K levels in breast cancer patients were assessed and compared to the control group (Figure 5). Low vitamin K levels were found in this study, which could be related to a lack of vitamin K supply or malabsorption, as well as drug interactions, and these low levels could exacerbate the disease stage. Importantly, researchers discovered that vitamin K intake has an inverse connection with overall cancer mortality (47). In addition to its critical role in blocking glycolytic enzymes and decreasing MCF-7 cell proliferation, vitamin K 3 has been demonstrated to have excellent cytotoxicity through the generation of reactive oxygen species (ROS) through mitochondrial disruption (48). The link between vitamin K and cancer, on the other hand, is currently unknown and under investigation. More research is needed to fully understand the role of vitamin K. Increased levels of SOD in breast cancer patients.

The limitation of our study must be small sample size, single center study, multiple vitamins need to be measured rather than only vitamin K, including vitamin E, vitamin C, and vitamin D (49, 50) or minerals (zinc) (51-53) which might involve in reducing oxidation, or other parameters of oxidative stress should be measured which might be as important as SOD, including arginase, metalloendopeptidase, peroxidase, myeloperoxidase, peroxynitrite, arylesterase and nitric oxide (54).

Conclusion

In conclusions, poor prognosis in cancerous cells might be a result of a complex mechanism derived from the effect of variant expression in NAD-dependent enzymes and low levels of Vitamin K, in addition to the disturbance of oxidant/antioxidant state. Collectively these findings might help with the understanding of the mechanisms that regulates cell proliferation or cancer cell functions and immune response in health and disease specifically in breast cancer. Measurement of other vitamins or oxidative stress-related biomolecules could be our approach for further studies.

Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee in the College of Sciences, University of Al-Qadisiyah, with approval number (3150 on 19.08.2021).

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