Effect of s-Klotho Protein, GPX, Visfatin, Leptin and ROS of Iraqi Women with Breast Cancer

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Abstract

   The most prevalent cancer is breast cancer, and the incidence of breast cancer in women worldwide is increasing at a remarkably rapid rate. This study was conducted on 90 samples (45 newly diagnosed breast cancer samples and 45 control group samples), ranging in age from 35 to 70 years. Blood samples were collected from the Alawia Teaching Hospital and the Oncology Teaching Hospital between October 2020 and March 2021. ELISA assessed ROS, GPX, visfatin, IGF-1, vitamin A, leptin, and soluble al-Klotho. The results indicate that the breast cancer patients had significantly higher (ROS 3.57, visfatin 17.44) (ng/mL) ($p < 0.0001$) and leptin 16.11 (ng/mL). In the group of patients, there was a significant increase ($p < 0.004$) compared with the control group, whereas there were no significant differences between IGF-1 (624.72 pg/mL) ($p > 0.507$), s-Klotho 398.22 ng/L ($p > 0.653$), and vitamin A 69.49 umol/L ($p < 0.05$). The results showed a negative correlation among the levels of patients with BC (ROS and vitamin A, GPX, IGF-1, GPX, leptin, and vitamin A). Finally, the current study concludes that vitamin A deficiency is one of the important factors that may influence the clinical evolution and prognosis of patients with breast cancer. Moreover, it was shown that the higher levels of leptin and visfatin could increase the danger in BC cases. The study measured levels of leptin and visfatin, which can increase the danger in BC cases, and measured ROS in patients.

Keywords: Breast cancer (BC), Visfatin, Leptin, soluble α-Klotho, Vitamin A, Reactive oxygen species.

تأثير بروتين s–klotho و GPX و Visfatin و Leptin و ROS للعراقيات المصابات بسرطان الثدي

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الخلاصة

   يعتبر سرطان الثدي أكثر أنواع السرطانات انتشارًا، كما أن معدل الإصابة بسرطان الثدي بين النساء في جميع أنحاء العالم يتزايد بمعدل سريع بشكل ملحوظ .أجريت هذه الدراسة على 90 عينة (45 عينة تم تشخيصها حديثًا بسرطان الثدي و 45 عينة مجموعة ضابطة)، تتراوح أعمارهم بين 35 و 70 عامًا. تم جمع عينات الدم من مستشفى العلوي التعليمي و مستشفى الأورام التعليمي بين أكتوبر 2020 و مارس 2021. تم تقييم انواع الاوكسجين التفاعلية وgpx و الفسفاتين وigf-1 و فتامين أ و الليبتين و الفا كلوثو الذائب من خلال تقنية

الاليزا. تشير النتائج إلى أن مرضى سرطان الثدي لديهم نسبة أعلى بشكل ملحوظ visfatin 3.57) ROS

17.44) انانوغرام / مل (p<0.0001) و اللبتين 16.11 (نانوغرام / مل). في مجموعة المرضى، كانت

هناك زيادة معنوية (0.004 > p) مقارنة بالمجموعة الضابطة، بينما لم تكن هناك فروق ذات دلالة إحصائية

بين 624.72) IGF−1) بيكوغرام / مل 398.22 s−Klotho (p>0.507) (نانوغرام (p>0.653) L و

فيتامين A 69.49 ميكرومول / لتر (p<0.05). أظهرت النتائج ارتباطًا سلبيًا بين مستويات مرضى BC

ROS) و فيتامين A و GPX و IGF−1 و GPX و leptin و فيتامين أ. اخيرًا، خلصت الدراسة الحالية إلى

أن نقص فيتامين أ هو أحد العوامل المهمة التي قد تؤثر على التطور السريري و التشخيص لمرضى سرطان

الثدي. علاوة على ذلك، فقد تبين أن المستويات الأعلى من اللبتين و الفيزفاتين يمكن أن تزيد من الخطر في

حالات سرطان الثدي حيث قامت هذه الدراسة بقياس مستويات اللبتين و الفيزفاتين، و التي يمكن أن تزيد من

الخطر في حالات سرطان الثدي ، و قياس انواع الاوكسجين التفاعلية في المرضى.

1. Introduction

Breast cancer is the world's second-most common cancer and the most common cancer in women [1,2]. Given the excessive occurrence, early identification and the adoption of more powerful drug treatments can lessen mortality and enhance the exceptional lifestyles of humans with this condition [3,4]. One of the trends associated with mitochondrial production is the manufacturing of reactive oxygen species (ROS) and sensitivity to ROS-caused apoptosis. Higher ROS degrees in non-converted cells or most cancer cells were said this way, and it's been recommended that accelerated ROS stages in non-converted cells or most cancer cells may also have pro-tumor results through unfavourable nucleic acids and inflicting genomic instability [5]. The catalytic location of glutathione peroxidase (GPX) consists of selenium and employs GSH as an electron donor to lower H_2O_2 to H_2O, changing glutathione disulfide (GSSG) to its oxidized form [6]. GSH is one of the most critical additives in the cell's antioxidant protection system. When GSH is conjugated to GPX, it aids in the removal of xenobiotics and carcinogens [7]. Visfatin is a 52-kDa adipokine found in visceral fats and different tissues that has an essential position in inflammation, metabolic and strain reaction variations, and mobile energy metabolism [8]. Visfatin has been implicated in the boom and/or metastasis of loads of malignancies lately [9]. Increased visfatin expression has been connected to the etiology of malignancies of the colon, brain, pancreas, liver, stomach, and prostate [10]. The Insulin-like Growth Factor 1 (IGF-1) pathway, which incorporates IGF-1, IGF-binding proteins (IGFBPs), and the IGF-1 receptor, is crucial to the improvement and function of several organs, including the mammary gland, in human physiology. IGF-1 is a critical mediator of mammary terminal stop buds and ductal development during growth [11]. In contrast, over the last few decades, numerous studies have linked the IGF-1 pathway to the development of a variety of cancers, including breast cancer [12,13]. Klotho is largely present in the female reproductive system of humans, namely the breast, ovary, and uterine tissues. During birth, it is mostly expressed in the placenta [14]. In some pregnancy issues, such as preeclampsia, irregular alpha-klotho expression has been discovered [15]. Breast cancer, cervical cancer, and epithelial ovarian cancer (EOC) are all hormonally linked malignancies [16]. Vitamin A merits special mention in this regard because of its important function in decreasing oxidative stress [17]. As well as its possible chemoprotective properties Because of vitamin A's antioxidant action, it has been linked to cancer treatments in the literature [18] The purpose of the study was to measure levels of leptin and visfatin, which can increase the danger in BC cases, and to measure ROS in patients.

2. Materials and methods

This study included 90 participants ranging, in age from 35 to 70 years old (45 newly diagnosed breast cancer patients and 45 healthy controls). Al-Eluia Health Facility for Female Care, a cancer coaching health center, furnished with blood samples. Every patient was subjected to a thorough physical examination. During the months of October 2020 to March 2021, the very last analysis changed to being made through aspirating cysts and checking cytology, histology (biopsy), and mammography. Following that, other biochemical indicators were assessed when the serum was separated. A human enzyme-linked immunosorbent assay (ELISA) kit (supplied from MyBioSource, USA, Cat No. MBS2515781, MBS167041, MBS723926,MBS765203, MBS729269,MBS020274, respectively), and s-Klotho by YH Biosearch laboratory was used to measure glutathione peroxidase (ng/mL), visfatin (ng/mL), insulin-like growth factor 1 (pg/mL), vitamin A (umol/L), leptin (ng/mL), and soluble Klotho (ng/L) from blood samples

3. Statistical analysis

The statistical analysis was carried out with the aid of the Statistical Package for Social Sciences laptop software version 20.0 for Windows (SPSS Statistic software). P values ≤ 0.05 were considered statistically significant. Data are expressed as mean $\pm$ standard error, and the mean standard error is used to express data
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4. Results

All data gathered was statistically analyzed and presented as a mean value with standard error. The P values were also included to verify differences in levels of study parameters between patients and controls. The results show biochemical parameters when comparing patients and control groups (Table 1). The patients with breast cancer had significantly higher values for ROS and visfatin (30.33, 27.39) (ng/mL) ($p<0.0001$), and leptin (20.18) (ng/mL) significant increase ($p<0.004$) compared to the control group, while no significant differences were found in IGF-1 (557.14) (pg/mL) ($p>0.507$) and s-Klotho (369.88) (ng/L) levels ($p>0.653$). Finally, it appears that the level of vitamin A (47.58 umol/L) in the patient group decreased significantly ($p<0.05$).

Table 1: Levels of biochemical parameters in patients with Breast cancer and control group

Variables	Sample	Number	Mean	S. E	P value
ROS (ng/mL)	Control	45	3.57	81	0.0001
	Patient	45	30.33	2.21	
GPX (ng/mL)	Control	45	251.22	7.52	0.0001
	Patient	45	85.67	3.27	
Visfatin (ng/mL)	Control	45	17.44	42	0.0001
	Patient	45	27.39	50	
IGF-1 (pg/mL)	Control	45	624.72	33.91	0.507
	Patient	45	557.14	95.63	
s-Klotho (ng/L)	Control	45	398.22	39.71	0.653
	Patient	45	369.88	48.58	
Vit A (umol/L)	Control	45	69.49	2.71	0.0001
	Patient	45	47.58	3.25	
Leptin (ng/mL)	Control	45	16.11	59	0.004
	Patient	45	20.18	1.25	

Pearson correlation analysis was used to investigate the relationship between all parameters covered in contemporary painting in terms of chemical measurements. The study discovered a negative correlation between the levels in BC patients (ROS and vitamin A, GPX and IGF-1, GPX and leptin, IGF-1 and vitamin A, as shown in Table 2.

Table 2: Correlations between variables in Breast cancer patients group (r value)

Variables	ROS	GPX	Visfatin	IGF-1	s-Klotho	Vitamin A	Leptin
ROS	1	-.011	.281	-.029	.155	-0.363[*]	.217
GPX	-.011	1	.024	-.456[**]	.016	.002	-.388[**]
Visfatin	.281	.024	1	.138	.005	-.051	.019
IGF-1	-.029	-.456[**]	.138	1	-.056	-.354[*]	.320[*]
s-Klotho	.155	.016	.005	-.056	1	.096	.442[**]
Vit A	-0.363[*]	.002	-.051	-.354[*]	.096	1	-.019
Leptin	.217	-.388[**]	.019	.320[*]	.442[**]	-.019	1

*. Correlation is significant at the 0.05 level. **. Correlation is highly significant at the 0.01 level.

5. Discussion

Alpha-Klotho was first identified as an anti-aging gene in 1997 [19]. These discoveries sparked a lot of interest in alpha-characteristic Klotho in ageing, and numerous studies have been conducted that linked Klotho loss or mutations to various age-related disease processes, primarily cardiovascular, renal, and musculoskeletal diseases [20]. In fact, new studies have showed that Klotho can play a role in the pathogenesis of human cancers [21-23]. Cancer is stated to be an age-associated case, with the occurrence of many cancers increasing with age [24]. The result was that there was no statistical significance between BC patients and the control groups. Rubinek *et al*. [25] analyzed Klotho expression using immunohistochemistry (IHC). They observed excessive expression of Klotho protein in ordinary tissue samples compared with decreased expression in odd ductal carcinoma [25]. Although Wolf *et al*. [26] were the first to discover decreased expression of klotho in IHC breast cancers and improved expression in all normal breast samples. In addition, about 90% of normal breast samples adjacent to tumor tissues [invasive ductal carcinoma or ductal carcinoma *in situ* (DCIS)], only 17% of DCIS tissues and 22% of invasive ductal carcinoma in situ tissues were identified [26]. Klotho steers the feature away from numerous signaling pathways associated with breast cancer tumorigenesis [27]. There may be few, if any, studies on the effect of s-Klotho on breast cancer in terms of serum levels. In this study, serum leptin and visfatin levels were studied, and the relationship between these adipokines and the risk of optic neuritis was examined. According to Assiri, Kamel and Hassanien [28], it was confirmed that serum levels of leptin and visfatin were statistically higher in patients with breast cancer compared with controls in this settlement. Leptin is also thought to affect the expression of the estrogen receptor (ER); however, other retrospective research suggests a negative relationship between leptin levels and BC risk [29,30]. Variability in pattern sizes, variability in pattern series and measuring methods, and heterogeneity of pre- and postmenopausal BC institutions can all contribute to contradictory leptin size results after diagnosis, as studies have shown that leptin and visfatin can increase the risk in BC cases. According to the findings, leptin may also be able to reduce BC risk in premenopausal women [31], as it may play a role in ovarian folliculogenesis, where follicular estradiol secretion may be decreased at higher levels [32]. Postmenopausal women with extended leptin tiers may be at a lower risk of BC due to leptin resistance and decreased leptin receptor activity [33]. Ghufran research has shown a lower level of nutrition at certain stages,

primarily when the most cancer levels are evolving, and this is related to our look at [34]. Elango, Samuel and Chinnakkannu proposed full-size nutrition discounts for most cancer patients, similar to their antioxidant function [35]. Some research has found no correlation between nutrition A attention and subsequent BC improvement [36]. Recent conflicting research on the relationship between IGF1 and breast cancer prevalence has revealed a fantastic correlation between IGF1 and breast cancer prevalence only in postmenopausal women [37]. Another study of 76 breast cancer recurrences in a group of 510 breast cancer survivors discovered no link between IGF1 and recurrence or death from the disease [38], as well as a beneficial correlation between IGF1 and recurrence of breast cancer among premenopausal African American women, which contradicts our findings. This conclusion is supported by a study conducted by Al-Jassani et al., which discovered a significantly higher level of serum ROS in patients while GPx was reduced compared to the control [39]. Antioxidant additives are depleted, resulting in decreased attention to a few antioxidants in patients suffering from oxidative stress [40].

6. Conclusion

The current study found a non-significant difference in serum s-Klotho levels when comparing patients with BC and control groups. The sample size should be increased, and other Iraqi provinces should be included in investigating Klotho's role as a tumor suppressor and a prognostic tumor biomarker with the ability to resource the early detection of malignancies. For the first time, we found no link between soluble klotho stages and Iraqi women with breast cancer. A larger sample size is required, necessitating additional research. Serum klotho may also function as a novel biomarker in patients with breast cancer patients. Vitamin A deficiency is one of the essential elements that could affect medical evolution and the analysis of patients with breast cancer.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

Conflict of interest

The authors declare that they have no conflict of interest.

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