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ALPHA-L-FUCOSIDASE AS A PUTATIVE PROGNOSTIC BIOMARKER IN BREAST CANCER

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Search for reliable biomarkers for predicting progression of breast cancer is essential in managing the disease. So, we are trying to provide new insights into the potential role of alpha-fucosidase (AFU) as a putative prognostic biomarker in breast cancer as compared to classic markers. The study included 56 women with breast cancer; 25 had early breast cancer, and the rest (31) had metastatic breast cancer. Thirty healthy women were considered a control group. Early breast cancer patients had a significantly increased (P ≤ 0.0001) AFU level compared with the control group. A non-significant difference in the De-ritis ratio appeared for early breast cancer compared with control. Metastatic breast cancer had a significantly (P ≤ 0.0001) increased AFU and De-ritis ratio compared with early breast cancer and the control group. A positive significant (P = 0.01) correlation exists between AFU level, age factor (r = 0.295), and the De-ritis ratio in breast cancer patients. We can conclude that it is possible to consider alpha-L-fucosidase (AFU) as a putative prognostic biomarker in breast cancer more potent than the ratio of De-Ritis. Moreover, the coincidence of elevated AFU and De-ritis levels in metastatic breast cancer gives us an idea of the stage of the disease.

K e y w o r d s: alpha-L-fucosidase, the De-ritis ratio, early breast cancer, metastasis.

The most prevalent malignancy diagnosed is breast cancer [1, 2] and the second leading cause of cancer mortality in females worldwide. It is a varied and heterogeneous disease with various symptoms, prognoses, and therapeutic responses [2].

The most frequent type of cancer in women globally is cancer of the breast [1]. Over 250,000 new cases were identified in 2017, indicating a lifetime incidence of 12% in women in the United States [3]. In the United States, over half of all breast cancer diagnoses are given to women over the age of 60. By 2040, the global burden of cancer will have increased by half compared to 2020 [4]. Furthermore, among Iraqi women, breast cancer ranks highest in incidence and mortality [5, 6]. The number of instances of breast cancer in Iraq rose from 26.6 per 100,000 in 2000 to 31.5 per 100,000 in 2009. The number of cases of breast cancer in Iraq rose from 26.6 per 100,000 in 2000 to 31.5 per 100,000 in 2009 [5, 7]. Iraq also has a higher age-related incidence rate than Iran, Bahrain, Saudi Arabia, and Turkey but lower rates than Kuwait and Jordan. Iraq also has a higher age-related incidence rate than Iran, Bahrain, Saudi Arabia, and Turkey but lower rates than Kuwait and Jordan [5]. In 2019, breast cancer ranked first in both incidence rate (35.95 per 100,000 people) and percentage of cancer cases; it also had the highest mortality rate (22.58 per 100,000 people) and incidence rate (34.08%) [8]. Metastasis is the leading cause of death among cancer patients. Circulating tumour cells must overcome numerous hurdles to infiltrate distant tissues. These include penetrating foreign tissue, avoiding immune defenses, adapting to supportive niches, living as dormant tumor-initiating seeds, and eventually erupting to replace the host tissue [9]. Breast cancer metastasis is still increasing in frequency. Cancer diagnostics can benefit from fucose related to tumor formation, lymphomagenesis, and vascular attack [10-12]. Many fucosylated glycans are degraded by alpha-L-fucosidase, which has long been known as a tumor marker linked to early diagnosis of H.C.C. as well as colon cancer [13-19]. Alpha-L-fucosidase (EC 3.2.1.51; AFU) is a lysoso-

mal enzyme. It removes terminal L-fucose residues from glycoconjugate oligosaccharide chains [17-25]. The AFU has been linked to the breakdown and disruption of alpha fucose levels in mammalian cells. As a result, an abnormality in the level of fucose isn't just related to an increase in the deformation of fucoglycones, such as glycoproteins, lipid molecules, and mucopolysaccharides found on the cell surface, but it is also linked to an alteration in the survival of cells. Cancer diagnostics can benefit from fucose related to tumor formation, lymphomagenesis, and vascular attack [10-12]. Because of the importance of AFU, the activity of Alpha-L-fucosidase has been investigated in a variety of sources [16, 21, 26-34].

The De-ritis ratio was first reported in 1957 by De-ritis and Gusi. Therefore, it has since been known as the De-ritis ratio [35]. De-ritis ratio represents the ratio of serum aspartate aminotransaminase (AST) to alanine aminotransaminase (ALT) [36, 37]. De-ritis ratio was used as a biomarker to predict viral hepatitis, but it is now used as a prognostic marker in many diseases, including hepatic cell carcinoma. (HCC) [38], breast carcinoma [39], renal cell carcinoma [40], testicular tumor [41], and urothelial carcinoma [42]. In some solid tumours, an elevated De-ritis ratio has been a poor diagnostic indicator [36, 42-44]. In prostate, renal, and urothelial carcinoma, a high De-ritis ratio has been reported to be a poor prognostic predictor [44]. A high De-ritis ratio has also been linked to a poor prognosis in other solid tumours, such as breast and lung cancer [36, 44].

In this study, we aimed to answer the following questions: 1 – Do metastasis breast cancer patients have a higher L-fucosidase and De-ritis than early breast cancer? 2 – Do patients correlate with the level activity of AFU and the level of De-ritis ratio (aspartate transaminase (AST)/alanine transaminase (ALT) ratio)?

Materials and Methods

Population study. The study was conducted on 56 patients with breast cancer aged (30-65) years. They attended the outpatient clinics of Al-Oram Teaching Hospital and the clinic for cancer disease licensed by the Ministry of Health in Mosul, Iraq. They were between December 15, 2020, and June 25, 2021. All patients diagnosed by specialists underwent a physical and clinical examination of the disease. In this study, patients with any other malignant tumours were excluded.

Also, 30 healthy women represented the control group, i.e. women with a healthy condition and without cancer. And their ages ranged from 30 to 65 years.

Experimental design. The selected samples were distributed into four groups as follows. The first group, 30 (Group 1), the second group, 56 (Group 2), included the total patients with breast cancer. The third group, 25 (Group 3), included women with breast cancer (the presence of a mass inside the breast) who had an early (initial) stage of progression of the disease. It was noted that there was swelling (swelling) of the glands under the armpit, with pain in the axillary area before treatment (some had it removed. They have new lumps. The four group 31 (Group 3) represented the metastatic breast cancer group in infection, as the disease was transmitted–metastasis to other organs, including the liver, lung, bone and spine.

All participants were given an informed consent questionnaire form to participate in the research. The study was carried out under the study protocol established by the Ethics Committee of the University of Mosul's College of Science and the Health Department in Nineveh (Protocol No. 20/153 on 23/12/2020).

Sample collection. Serum The AFU level was measured four weeks before the procedure. Patients fasted, venous blood samples were taken, and serum was extracted by centrifuge [12, 45].

Five millilitres of Fasting venous blood samples were collected from adults. After coagulation for 20 min at 37°C, it was centrifuged at 3000 g for 20 min. Serum was collected and stored at -20°C [12].

Serum samples were collected from patients with early breast cancer before surgical removal of the primary tumour, with no neoadjuvant treatment or indication of metastasis.

The serum samples for the metastatic breast cancer patients were obtained after at least one confirmed metastasis, with a median period of 12 months from about the date of the first metastasis.

Assay of α-L-fucosidase level. The sandwich-ELISA method was employed to assay the level of α-Lfucosidase. by using of the E-EL-H0290 Human FUCA (alpha-L-fucosidase, Tissue) ELISA Kit from Elabscience. It is also known as FUCA1, A-FU, or aFU. The manufacturer's instructions were followed when the test was conducted, and the idea of the test was incorporated. This kit's micro ELISA plate al-

ready has a human FUCA-specific antibody. Before combining the sample or standard with the appropriate antibody, they add it to the wells of the micro ELISA plate. A biotinylated detection antibody specific to human FUCA is applied to each well of the microplate, followed by an Avidin-Horseradish Peroxidase (HRP) conjugate, and the plates are then incubated. Dissolved components are removed by washing. Every well is then filled with the substrate solution. A blue colour will be produced only in the wells that contain the Human FUCA, biotinylated detection antibody, and Avidin-HRP conjugate. The hue changes to yellow when the enzyme-substrate reaction is stopped by adding the stop solution. At a wavelength of 450 ± 2 nm, the spectrophotometric measurement of the optical density (OD) is taken. The concentration of Human FUCA is directly related to the OD value. By comparing the samples' optical density (OD) with the standard curve, you may determine the concentration of human FUCA in the samples. Before testing, a one-to-five ratio was used to dilute all samples in a diluted solution.

Assay the level of De-ritis ratio (AST/ALT ratio). According to the manufacturer's instructions, each AST and ALT was measured using the Japanese-origin Fuji self-analysis device [46]. Then, the De-ritis ratio (AST/ALT ratio) was calculated by dividing the AST result by the ALT result [47].

Statistical analysis. The analysis was carried out using SPSS version 25. They examined the data. After obtaining the mean ± standard deviation (SD), an independent T-test was used to compare the two groups. Pearson's correlation coefficient was used to express the results of the correlation investigation. The *P*-value lower than 0.05 was considered statistically significant [48, 49]

Results and Discussion

Effect of the age factor on breast cancer. Table 1 shows the mean ± SD of the age of breast cancer patients. Each breast cancer case has a matched age (58.6 ± 10 years) and controls (54.6 ± 11 years). Therefore, there is not a significant ($P = 0.897$). Also, there are non-significant differences ($P = 0.06$) between patients with early-stage breast cancer (50.1 ± 6.2 years) and control (54.6 ± 11 years). At the same time, there is a significant difference ($P \leq 0.0001$) appeared for age of patients with metastatic breast cancer (65.9 ± 5.8 years) when compared with each of the control group (54.6 ± 12.8 years) and early-stage breast cancer group (50.1 ± 65.2 years). These results mean that risk of breast cancer progression increases with advanced age [4, 50].

Level of alpha-L-fucosidase (AFU) in patients with breast cancer. Table 2 show the level of AFU in breast cancer patients. Significantly elevated AFU levels in the serum of breast cancer patients (22.7 ± 9.8 ng/ml; $P \leq 0.0001$) compared with the healthy control group (6.3 ± 2.6 ng/ml). A significant increase ($P \leq 0.05$) in the level of AFU in early breast cancer (11.9 ± 3.6 ng/ml) compared with the healthy controls (6.3 ± 2.6 ng/ml). The level of AFU is significantly increased ($P \leq 0.001$) for metastatic breast cancer (33.6 ± 5 ng/ml) when compared with both early breast cancer (11.9 ± 3.6 ng/ml) and the healthy group (6.3 ± 2.6 ng/ml). These results indicate an increasing AFU level with tumor progression. Several studies have found that alpha-L-fucosidase activity is increased in cancer patients. They note that the level of AFU can be used for prognosis of the course of the disease and its outcome [11, 12, 30, 51]. The cause of the rise in alpha-L-fucosidase remains unknown. One probable explanation is that the tumor produces more proteins, which increases fucose metabolism [14]. Increased fucose or fucosylation content and aberrant fucosylation on the surface of the membrane of tumor cells may be attributed to tumor distant metastasis capacity. Also, it could assist abnormal cells in escaping immunological recognition [30, 45, 52, 53].

Level of De-ritis ratio (AST/ALT ratio) in breast cancer patients. The results in Table 3 showed breast cancer patients' De-ritis ratio (AST/ALT ratio) level. Significant increase in the rate of De-ritis for breast cancer patients (1.5 ± 0.7; $P \leq 0.0001$) compared with the healthy control group (0.68 ± 0.15). In contrast, there was no significant difference ($P = 0.06$) in the level of De-ritis for early breast cancer (0.84 ± 0.37) compared with healthy controls (0.68 ± 0.15). However, there was a significant increase ($P \leq 0.0001$) in the De-ritis ratio for patients with metastatic breast cancer (2 ± 0.32) when compared with early breast cancer patients (0.84 ± 0.37) and healthy subjects (0.68 ± 0.15). According to our findings, the ratio of De-ritis can be considered an independent prognostic factor for breast cancer progression. Several studies have indicated that the rate of De-ritis is an important prognostic indicator for many types of cancer [36, 38, 40-42, 44, 54].

The elevated AST/ALT ratio in cancer patients is still a mystery. The "Warburg effect" has revealed that most cancer cells use anaerobic glycolysis to

Table 1. Age of patients with metastatic and early stage breast cancer

Cases	*n*	Age (years), mean ± SD	*P*-Value
Control group	30	54.6 ± 11.0	0.897
Breast cancer	56	58.6 ± 10.0	0.897
Control group	30	54.7 ± 11.0	0.06
Early stage breast cancer	25	50.1 ± 6.2	0.06
Control group	30	54.6 ± 11.0	0.0001
Metastatic breast cancer	31	65.9 ± 5.8***	0.0001
Early stage breast cancer	25	49.7 ± 6.2	0.000
Metastatic breast cancer	31	65.9 ± 5.8	0.000

Note. *** refer to the *P*-value higher a significant at $P \leq 0.0001$

Table 2. Level of alpha-L-fucosidase (AFU) in patients with breast cancer

Cases	*n*	AFU (ng/ml), mean ± SD	*P*-Value
Control group	30	6.3 ± 2.6	0.0001
Breast cancer	56	23.9 ± 11.5***	0.0001
Control group	30	6.3 ± 2.6	0.041
Early stage breast cancer	25	11.9 ± 3.6*	0.041
Control group	30	6.3 ± 2.6	0.0001
Metastatic breast cancer	31	33.6 ± 5***	0.0001
Early stage breast cancer	25	11.9 ± .4	0.0001
Metastatic breast cancer	31	33.6 ± 5***	0.0001

Note. *Refer to the *P*-value a significant at $P \leq 0.05$; ***refer to the *P*-value a higher significant at $P \leq 0.0001$

Table 3. Level of the De-ritis ratio (AST/ALT ratio) in breast cancer patients

Cases	*n*	AST/ALT, mean ± SD	*P*-Value
Control group	30	0.68 ± 0.15	0.0001
Breast cancer	56	1.5 ± 0.7***	0.0001
Control group	30	0.68 ± 0.17248	0.06
Early stage breast cancer	25	0.84 ± 0.37	0.06
Control group	30	0.68 ± 0.15	0.0001
Metastatic breast cancer	31	2.00 ± 0.32***	0.0001
Early stage breast cancer	25	0.84 ± 0.37	0.0001
Metastatic breast cancer	31	2.0 ± 0.32***	0.0001

Note. *Refer to the *P*-value a significance at $P \leq 0.05$; ***: refer to the *P*-value a higher significant at $P \leq 0.0001$. AST – alanine aspartate transaminase; ALT – alanine transferase

generate the energy needed for cellular growth, survival, as well as metastases, even in the presence of oxygen [55-57]. AST and ALT enzymes are related to glutamine metabolism, which is essential for cancer cells to produce nucleotides and nonessential amino acids [58-60]. The enzymes AST and ALT, which represent tumour metabolism, serve as biomarkers to predict patient prognosis [42, 54, 61]. The elevated AST/ALT ratio in cancer patients is still a mystery. The "Warburg effect" has revealed that most cancer

Table 4. Correlation of AFU level with the De-ritis ratio in breast cancer patients

Parametrs	Groups		
	Total patients BC	Early stage BC	Metastatic BC
	AFU, ng/ml		
Pearson correlation	0.89**	0.79**	0.89**
P-value	0.01	0.01	0.01
n	56	31	25
	De-ritis ratio		
Pearson correlation	0.96**	0.32	0.83**
P-value	0.01	0.01	0.114
n	56	31	25

Note. **Refer to the *P*-value a higher significance of a correlation at $P \leq 0.01$; BC – breast cancer; AFU – alpha-L-fucosidse

Table 5. The area for the variable studies in breast cancer using receiver operating characteristic (ROC) curve analysis

Test result variable(s)	Groups		
	Total patients BC	Early stage BC	Metastatic BC
AFU, ng/ml	1.000	0.492	1.000
De-ritis ratio	0.872	0.360	0.991
Age, years	0.602	0.182	0.885

cells use anaerobic glycolysis to generate the energy needed for cellular growth, survival, and metastases, even in oxygen [55-57]. AST and ALT enzymes are related to glutamine metabolism, essential for cancer cells to produce nucleotides and nonessential amino acids [58-60]. Therefore, AST and ALT, representing tumour metabolism, act as biomarkers to predict disease prognosis [42, 54, 61].

Correlation of AFU level with De-ritis ratio in breast cancer patients. The results in Table 4 revealed a positive significant correlation ($P = 0.01$) for AFU level with each age for all patients with breast cancer ($r = 0.88$), early-stage breast cancer ($r = 0.79$) and metastatic breast cancer ($r = 0.89$). Also, the results in Table 4 revealed a positive significant correlation ($P = 0.01$) for AFU level with each and the De-ritis ratio for all patients with breast cancer ($r = 0.96$) and metastatic breast cancer ($r = 0.83$). At the same time, there is a non-significant correlation at early-stage breast cancer ($r = 0.32$) for AFU level with the De-ritis ratio.

The reason for appearing a significant correlation between AFU and age in all stages of breast cancer may be that breast cancer disease is a progression with advanced age [4, 50]. The age factor also has an impact on the level of AFU.

Also, the significant positive correlation between AFU activity and the De-ritis ratio may be due to an increase in the enzyme activity associated with the disease's progress, especially in the case of metastatic breast cancer. In metastatic cancer, the tumour cells need glycolysis to produce more energy [55-57].

Conclusion. We can conclude that it is possible to consider alpha-L-fucosidase a tumor marker in the early diagnosis of breast cancer more than the De-ritis ratio. Moreover, the coincidence of elevated AFU and De-Ritis levels in metastatic breast cancer gives us an idea of the stage of the disease.

Age is one of the most critical factors that can increase breast cancer risk. A significant positive correlation between the level of AFU activity and increased age may be because most patients with metastatic breast cancer are about years older than those with early breast cancer.

Also, the significant positive correlation between AFU activity and the De-ritis ratio may be due to an increase in the enzyme activity associated

with the disease's progress, especially in the case of metastatic breast cancer. In metastatic cancer, the tumor cells need glycolysis to produce more energy. Also, the alpha-L-fucosidase may be a good tumor marker for diagnosing breast cancer, especially in the metastasis stage, with and combined assay with the De-ritis ratio and the age factor. Moreover, ROC analysis shows that the alpha-L-fucosidase may be considered a promising tumor marker for diagnosing breast cancer, especially in the metastasis stage, with and combined assay with the De-ritis ratio and the age factor.

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АЛЬФА-L-ФУКОЗИДАЗА ЯК ПЕРЕДБАЧУВАНИЙ ПРОГНОСТИЧНИЙ БІОМАРКЕР ПРИ РАКУ МОЛОЧНОЇ ЗАЛОЗИ

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Пошук надійних біомаркерів для прогнозування перебігу раку молочної залози має важливе значення для лікування захворювання. Метою дослідження було оцінити потенційну роль альфа-фукозидази (AFU) як передбачуваного прогностичного біомаркера розвитку раку молочної залози порівняно з класичними маркерами. У дослідженні взяли участь 30 здорових жінок (контрольна група) і 56 жінок з раком молочної залози, серед яких 25 пацієнток мали ранній і 31 – метастатичний рак молочної залози. Показано, що у пацієнток із ранніми стадіями раку молочної залози рівень AFU був суттєво підвищений, а коефіцієнт Де Рітіса достовірно не відрізнявся від контрольної групи. Пацієнтки з метастатичним раком молочної залози мали достовірно підвищені значення як AFU, так і коефіцієнта Де Рітіса порівняно з пацієнтками з ранньою стадією раку молочної залози та контрольною групою. Встановлена позитивна достовірна кореляція між рівнем AFU, віковим фактором (r = 0,295) та коефіцієнтом Де Рітіса у пацієнток із раком молочної залози. Зроблено висновок, що α-L-фукозидазу можна вважати передбачуваним прогностичним біомаркером, більш вагомим порівняно з коефіцієнтом Де Рітіса при раку молочної залози. До того ж наявність підвищених рівнів AFU та коефіцієнта Де Рітіса дає нам певне уявлення про стадію захворювання.

Ключові слова: альфа-L-фукозидаза, коефіцієнт Де Рітіса, ранній рак молочної залози, метастази.

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