Alfarama Journal of Basic & Applied Sciences

Faculty of Science Port Said University

https://ajbas.journals.ekb.eg
ajbas@sci.psu.edu.eg
http://sci.psu.edu.eg/en/

July 2024, Volume 5, Issue III

DOI: 10.21608/AJBAS.2024.292265.1214

ISSN 2682-275X

Submitted: 09/06/2024
Accepted: 15/06/2024

Pages: 395 – 408

Study of the Role of Trefoil Factor Family3 protein level as Molecular Marker for Early Detection of Endometrial Carcinoma Micrometastasis

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ABSTRACT

Background and objectives: Endometrial carcinoma is the predominant gynecologic cancer in industrialized nations and the second most prevalent in developing nations, following cervical carcinoma. Serum biomarkers have the potential to be utilized in the screening, diagnosis, prognosis, and therapy monitoring of endometrial cancer micrometastasis. Trefoil family 3 (TFF3) is known to be excessively produced as a protein in various types of human tumors, such as intestinal, pancreatic, and prostate cancers. It is also implicated in the advancement of gastric cancer. Furthermore, the level of the TFF-3 protein in breast cancer is regularly controlled by estrogen, and similar associations have also been observed in endometrial cancer.

Subjects and methods: This case-control done in Mansoura University, Egypt on a total of 75 patients with 25 age-matched healthy female volunteers as control group and 50 patients were classified according to standard criteria based on data of International Federation of Gynecology and Obstetrics (FIGO) into two groups (non-metastatic endometrial carcinoma and metastatic endometrial carcinoma). Total RNA was extracted from peripheral blood, Real-time PCR for quantification of TFF3 and Serum samples was used to measure TFF3 protein level by ELISA.

Results: Comparison of the control group, patients with non-metastatic endometrial cancer, and patients with metastatic endometrial carcinoma in terms of TFF3 protein level revealed highly significant differences ((0.41 ± 0.15), (3.79 ± 3.05) and (6.09 ± 3.64)) respectively, P-values (all< 0.001). AUC values for TFF3 protein level equal 0.654 while TFF3 protein level showed sensitivity percentage of 72%

and 60% specificity percent. In the univariable analysis TFF3 protein level gave an odds ratio (ORs) 1.232 at p-values of 0.031while in the multivariable analysis demonstrated an odds ratio (1.212), p-values (0.019).

Keywords:

endometrial carcinoma, gene expression, TFF3 protein level.

1. INTRODUCTION

Endometrial carcinoma is the predominant gynecologic cancer in industrialized countries and the second most prevalent in under developed countries, following cervical carcinoma. It has a favorable prognosis because it is usually diagnosed early by abnormal uterine bleeding especially in postmenopausal women. However, in premenopausal women, this uterine bleeding may not be related to menstrual cycle or long heavy bleeding during menstrual cycle may occurs [1]. Endometrial Carcinoma initially metastasizes to the myometrium and serosa before spreading to additional reproductive and pelvic systems. The lymphatic system typically involves the pelvic and para-aortic nodes as the initial sites of involvement. Distant metastases occur by the blood often to lungs, liver, brain and bone [2]. Metastasis is a multifaceted process comprising a sequence of intricate processes. The process commences with the invasion of adjacent host tissue by cells originating from the primary tumor. Subsequently, cells migrate into the circulation or lymphatic pathways and disseminate to far organs. They adhere to the capillary beds of the target organ. Afterwards, they penetrate the tissue of the organ and rapidly reproduce, triggering the development of new blood vessels within the organ. During these stages, the tumor cells need to elude the immune response of the host and prevent themselves from undergoing programmed cell death (apoptosis) in order to guarantee their survival [3]. Circulating tumor cells (CTCs) refer to the cells that have been released from the main tumor and are now present in the bloodstream. They are transported through the bloodstream to other organs in the body, resulting in metastasis. CTCs serve as the initial source for the development of secondary cancers (metastases) in distant organs. Classical imaging techniques frequently fail to detect micrometastases [4]. The identification of distinct molecular indicators on circulating tumor cells (CTCs) in the bloodstream can accurately forecast the patient's prognosis. Endometrial carcinoma [5] exhibits detectable elevation in the expression level of several markers. One of these indicators is the trefoil factor family three (TFF3). TFF3 belongs to the trefoil factor family, which is distinguished by the presence of a three-loop structure known as the trefoil domain. The formation of this trefoil domain is facilitated by the disulfide connections that connect cysteine residues [6].

TFF3 is typically expressed in the gastrointestinal tract (GIT) and functions in the defense against mucosal injury. This function is linked to mucin, specifically TFF3. TFF3 and mucin collaborate to form a resilient gelatinous coating of mucus. The gastrointestinal mucosa is protected and repaired by this layer, which plays a vital part in the process. Nevertheless, TFF3 possesses oncogenic properties by promoting cell proliferation, inhibiting apoptosis, enhancing cell migration, invasion, and facilitating new blood vessel formation [7].CTCs have a prospective role since they can function as a liquid biopsy for the early diagnosis of cancer metastases [8]. The objective of this study was to investigate the role of TFF3 protein levels as a molecular marker for early detection of micrometastases in endometrial carcinoma. Additionally, the study aimed to explore the feasibility of using blood samples as a non-invasive alternative to invasive biopsies or costly imaging techniques for detecting micrometastases in endometrial carcinoma.

2. STUDY AREA

Arab Republic of Egypt.

3. SUBJECTS AND METHODS

Patients and controls

This study included 50 cases of female patients and 25 control subjects matched in age. The mean age of non-metastatic and metastatic groups was:(49.0-73.0) and (48.0-83.0) years, respectively. Patients will be classified according to standard criteria based on data of international federation of Gynecology and Obstetrics (FIGO) staging systems into two groups: The first group: 25 cases of non-metastatic endometrial carcinoma (stage I-II). The second group: 25 cases of metastatic endometrial carcinoma (stage III-IV). all collected randomly from the oncology Center, Mansoura University Hospital, in the period from December 2019 to February 2022.

Sampling and management of specimens

A 5 mL sample of peripheral venous blood was taken from individuals using EDTA tubes, properly labeled, transferred on ice to Medical Biochemistry Department and stored at -20 º C until processing. Another blood sample collected in anti-coagulant free tubes and left at room temperature for 20 minutes and then centrifuged to separate serum and stored at -20 º C until processing.

Laboratory analysis

Circulating tumor cells will be separated by density- gradient centrifugation through Ficoll, Total RNA will be extracted from peripheral blood, RNA concentration and purity will be assayed, cDNA will be synthesized from RNA, Primer design will be done and primer conditioning will be done using PCR, Real-time PCR for quantification of TFF3 and Serum samples will be used to measure TFF3 level by ELISA.

Statistical analysis:

The data that has been gathered will be condensed, organized into tables, and examined using SPSS software version 21. IBM Corp. released in 2017.The software used is IBM SPSS Statistics for Windows, specifically Version 25.0. It is developed by IBM Corp. and is based in Armonk, NY. The suitable statistical tests employed for data analysis. A P value below 0.05 is deemed statistically significant.

4. RESULTS AND DISCUSSION

Results:

Table 1 presents a comprehensive comparison of demographic and anthropometric data among three different groups: Group I (Control), Group IIA (Non-metastatic endometrial carcinoma), and Group IIB (Metastatic endometrial carcinoma) .

The study reveals that there is no statistically significant difference in age among the three groups, as evidenced by the p-value being greater than 0.05. This implies that the age distribution is comparable among these groups. Nevertheless, there is a quite substantial disparity in weight across the groups ($p < 0.001$), with Group IIB (Metastatic endometrial carcinoma) having the highest mean weight, followed by Group IIA (Non-metastatic endometrial carcinoma) and Group I (Control). Post hoc tests reveal that the differences in weight are significant between all pairs of groups except for the comparison between Group IIA and Group IIB, where the p-value >0.05.For the number of pregnancies, There was no statistically significant difference seen among the three groups, as shown by a p-value greater than 0.05. Similarly, when comparing the ratio of individuals who have given birth (parous) and those who have not (nulliparous), there are no statistically significant variations across the categories.

Table 1. Presents a comparison of demographic and anthropometric data for the three groups that were evaluated.

	Group I n = 25	Group IIA n = 25	Group IIB n = 25
Age (years)			
Mean ± SD.	61.48 ± 7.98	61.92 ± 6.83	64.80 ± 7.67
Median (Min. – Max.)	62.0(48.0 – 75.0)	62.0(49.0 – 73.0)	65.0(48.0 – 83.0)
Weight (kg)			
Mean ± SD.	75.76 ± 9.20	93.24 ± 7.92	97.56 ± 6.12
Median (Min. – Max.)	75.0 (57.0 – 91.0)	95.0 (75.0 – 110.0)	99.0 (88.0 – 110.0)
Number of pregnancy			
Mean ± SE.	2.52 ± 0.28	2.76 ± 0.37	3.36 ± 0.34
Median (Min. – Max.)	3.0 (0.0 – 5.0)	3.0 (0.0 – 6.0)	4.0 (0.0 – 6.0)
Parous	23 (92.0%)	21 (84.0%)	23 (92.0%)
Nulliparous	2 (8.0%)	4 (16.0%)	2 (8.0%)

Group I: Control, Group IIA: Non-metastatic endometrial carcinoma, Group IIB: Metastatic endometrial carcinoma

*:Significant ≤0.05

Table 2. An analysis of three groups was conducted to compare their menopause status.

	Group I n = 25		**Group IIA n = 25**		**Group IIB n = 25**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Menopause status						
Pre-Menopause	4	16.0	2	8.0	0	0.0
Menopause	21	84.0	23	92.0	25	100

Group I: Control, Group IIA: Non-metastatic endometrial carcinoma, Group IIB: Metastatic endometrial carcinoma

Table 2 presents a detailed comparison of menopause status among three distinct groups: Control group, Non-metastatic endometrial carcinoma group and Metastatic endometrial carcinoma group. The analysis reveals that there is no difference in menopause status distribution among the three groups, Metastatic endometrial carcinoma group having a higher percentage of individuals in the "Menopause" category compared to Control group and Non-metastatic endometrial carcinoma group. Nevertheless, this discrepancy does not have a substantial statistical impact (p1 >0.05). Additional post hoc tests were undertaken to investigate pairwise comparisons. These tests reveal that there is no statistically significant difference between Control group and Non-metastatic endometrial carcinoma group, Control group and Metastatic endometrial carcinoma group, or between Non-metastatic endometrial carcinoma group and Metastatic endometrial carcinoma group.

Table 3. Comparison of endometrial carcinoma groups with and without metastasis regarding stage and primary tumor size.

	Group IIA n = 25		**Group IIB n = 25**	
	No.	**%**	**No.**	**%**
Stage				
I	14	56.0	0	0.0
II	11	44.0	0	0.0
III	0	0.0	13	52.0
IV	0	0.0	12	48.0
Primary tumor size (cm)				
<5 cm	16	64.0	12	48.0
>5 cm	9	36.0	13	52.0

Group IIA: Non-metastatic endometrial carcinoma, Group IIB: Metastatic endometrial carcinoma

Table 3 presents a comparison between two groups of endometrial carcinoma patients, non-metastatic group and metastatic group, in terms of their cancer stage and primary tumor size. Group of Non-metastatic endometrial carcinoma includes 25 patients, primarily in Stage I and II, with 56% at Stage I and 44% at Stage II. None of the Group IIA patients is in Stage III or IV. Group of Metastatic endometrial carcinoma (consisting of 25 patients) falls into Stage III (52%) and Stage IV (48%), with no patients in Stage I or II. When examining the size of the first tumor, there is no notable distinction between the two groups. Specifically, 64% of Group IIA and 52% of Group IIB had tumors that were smaller than 5 cm.The statistical test (p>0.05) indicates that there is no statistically significant difference in tumor size between the groups.

Table 4. Distribution of metastatic endometrial carcinoma group regarding to metastatic sites.

	No.	**%**
Metastatic site		
Bone	4	16.0
Lung	7	28.0
Pelvic	7	28.0
Peritoneum	7	28.0

Table 4 presents data on the distribution of metastatic sites in the endometrial carcinoma group. It shows that metastasis in this group is quite diverse, with the highest frequency of cases occurring in the

lung, pelvic region, and peritoneum, each accounting for 28% of the cases. Meanwhile, bone metastasis is less common, comprising only 16% of the cases. These findings highlight the propensity of endometrial carcinoma to spread to various anatomical sites, with a preference for intrapelvic and intraperitoneal locations.

Table 5. Comparison of three studied groups regarding the TFF3 protein level

	Group I n = 25	**Group IIA n = 25**	**Group IIB n = 25**
TFF3 protein level			
Mean ± SD.	0.41 ± 0.15	3.79 ± 3.05	6.09 ± 3.64
Median (Min. – Max.)	0.4 (0.2 – 0.8)	2.5 (1.0 – 10.0)	7.6 (0.7 – 10.0)

Group I: Control, Group IIA: Non-metastatic endometrial carcinoma, Group IIB: Metastatic endometrial carcinoma,

*: Significant ≤0.05

The table 5 provides a comprehensive comparison of three distinct groups: Group I, representing the control group; Group IIA, comprising patients with non-metastatic endometrial carcinoma; and Group IIB, consisting of individuals with metastatic endometrial carcinoma.The focus of this comparison lies in evaluating key parameter TFF3 protein levels.The findings reveal highly significant differences among all three groups for each of the parameters examined. The p-values (all <0.001) suggest that these differences are indicative of real disparities in protein levels among the groups. Upon conducting post hoc tests, we have observed that the levels of TFF3 protein in Group IIA are significantly greater than those in the control group. However, it is in Group IIB that we have noticed a significant difference compared to both Group I and Group IIA ($p<0.05$). This implies that the levels of TFF3 protein may not only indicate the advancement of metastasis, but also have the capacity to differentiate between instances that are non-metastatic and cases that are metastatic.

Table (6). Validity of TFF3 protein level for discrimination between healthy subjects and patients with endometrial carcinoma.

	TFF3 protein level
AUC	0.999
95% CI	0.997 – 1.00
P	<0.001*
Cut off	>0.688
Sensitivity (%)	98
Specificity (%)	100

PPV (%)	100.0
NPV (%)	96.2
Accuracy (%)	98.7

AUC, area under ROC curve; CI, confidence interval;

PPV, positive predictive value; NPV, negative predictive value.

*: Significant ≤0.05

Table 6 provides crucial insights into the validity of using TFF3 protein levels, as diagnostic markers to discriminate between healthy subjects and patients with endometrial carcinoma.The data indicates remarkably high area under the ROC curve (AUC) values for parameter, with AUC values of 0.999 for TFF3 protein levels. These AUC values suggest that amarker are excellent at distinguishing between healthy individuals and those with endometrial carcinoma. Additionally, high sensitivity and specificity percentages reinforce their effectiveness as diagnostic tools, TFF3 protein levels (98% sensitivity and 100% specificity).The positive predictive values (PPV) and negative predictive values (NPV) further highlight the accuracy of this marker in predicting disease presence or absence. This underscores the high diagnostic potential of TFF3 protein levels in identifying endometrial carcinoma, making it valuable candidates for clinical use in early detection and patient management.

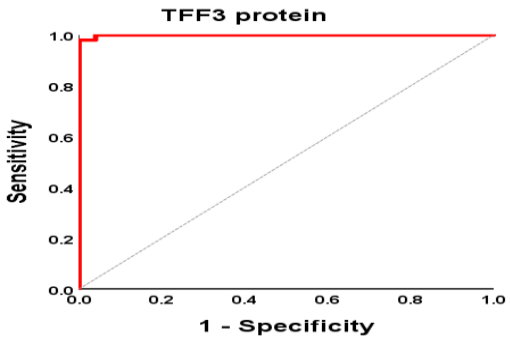

Figure (1). ROC Curve of TFF3 protein level for discrimination between healthy subjects and patients with endometrial carcinoma.

Table (6). Validity of TFF3 protein level for discrimination between non-metastatic and metastatic endometrial carcinoma.

	TFF3 protein level
AUC	0.654
95% CI	0.494 – 0.815
P	0.061
Cut off	>2.74
Sensitivity (%)	72
Specificity (%)	60
PPV (%)	64.3
NPV (%)	68.2
Accuracy (%)	66

AUC, area under ROC curve; CI, confidence interval;

PPV, positive predictive value; NPV, negative predictive value.

*: Significant ≤0.05

Table 6 provides valuable insights into the validity of using TFF3 protein levels, as discriminative markers for distinguishing between non-metastatic and metastatic endometrial carcinoma cases.low AUC was found with TFF3 protein levels at 0.654.Sensitivity and specificity percentages are low with TFF3 protein levels showing (72%) for Sensitivity and (60%) for Specificity, So TFF3 protein levels contribute as a particularly promising marker for differentiating between non-metastatic and metastatic endometrial carcinoma cases but to a lesser extent.

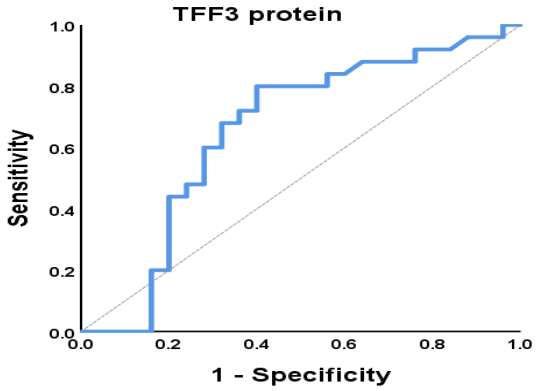

Figure (2). ROC Curve of TFF3 protein level between non-metastatic and metastatic endometrial carcinoma.

Table 8. Correlation between TFF3 protein level with different parameters among all studied subjects.

	TFF3 protein level	
	r	***P***
Age	0.014	0.907
Weight	0.500	<0.001*
No. of pregnancy	0.124	0.289

r, correlation coefficient

Table 8 presents the correlation between TFF3 protein level, and various parameters among all studied subjects.There is a positive correlation between TFF3 protein level and body weight (r=0.500, p<0.001*), suggesting that individuals with higher body weights tend to have elevated TFF3 protein levels.The age and number of pregnancies does not exhibit a significant correlation with TFF3 protein levels (p>0.05).

Table (9). Logistic Regression analysis for prediction of endometrial carcinoma susceptibility.

	Univariable			**Multivariable**		
	p	**OR**	**95% C.I**	**P**	**OR**	**95% C.I**
Age	0.309	1.034	0.969 – 1.104			
Weight	<0.001*	1.325	1.163 – 1.510	0.092	1.012	0.946-1.018
Nulliparous	0.599	1.568	0.293 – 8.396			
Menopause status	0.093	4.571	0.776 – 26.92			
TFF3 protein	0.031*	1.232	1.019 – 1.489	0.019*	1.212	1.164-2.032

OR, odds ratio; CI, confidence interval.

*: Significant ≤0.05

Table 9 provides valuable insights into the logistic regression analysis aimed at predicting susceptibility to endometrial carcinoma. In the univariable analysis, several variables were examined for their individual associations with endometrial carcinoma risk.Among these variables, weight stands out with a significant p-value (<0.001) and an odds ratio (OR) of 1.325, indicating that higher body weight is strongly associated with an increased risk of endometrial carcinoma.TFF3 protein levels exhibit a significant association with a p-value of 0.031 and an OR of 1.232.In the multivariable analysis, which considers multiple variables simultaneously, weight lost the significance (p=0.092).Notably, TFF3 protein levels, maintain their significance (p=0.019) and demonstrate elevated ORs.These findings highlight the potential utility of TFF3 protein level as independent predictor of endometrial carcinoma susceptibility. The results emphasize the importance of this molecular marker in assessing individual risk and may have implication for preventive strategies and early intervention in endometrial carcinoma.

Table (10). Logistic Regression analysis for prediction of metastatic endometrial carcinoma.

	Univariable			Multivariable		
	P	OR	95% C.I	p	OR	95% C.I
Age	0.168	1.058	0.976 – 1.147			
Weight	0.044*	1.094	1.002 – 1.195	0.476	1.102	0.844 – 1.438
Nulliparous	0.393	0.457	0.076 – 2.755			
Menopause stage	0.999	-	-			
Primary tumour size	0.257	1.926	0.621 – 5.977			
TFF3 protein	0.021*	1.002	1.0 – 1.004	0.629	1.001	0.997 – 1.005

OR, odds ratio; CI, confidence interval.

*: Significant ≤0.05

Table 10 provides valuable insights into logistic regression analysis aimed at predicting the risk of developing metastatic endometrial carcinoma. In the univariable analysis, several variables were examined for their individual associations with metastatic endometrial carcinoma risk. Among these variables, weight emerges as significant with a p-value of 0.044 and an odds ratio (OR) of 1.094, suggesting that higher body weight is associated with an increased risk of metastatic endometrial carcinoma. TFF3 protein levels exhibit a significant association with a p-value of 0.021 and an OR close to 1, indicating that elevated expression level of this gene are linked to a higher risk of metastasis.In the multivariable analysis, which considers multiple variables simultaneously, weight loses its significance (p>0.05), suggesting that its association with metastatic endometrial carcinoma risk may be influenced by other factors in the model. Interestingly, TFF3 protein levels do not remain significant in the multivariable analysis.

5. DISCUSION

Uterine corpus cancer is the most common type of cancer affecting the female reproductive system in American women. It is estimated that there will be over 60,000 new cases in the coming year, resulting in over 11,000 fatalities.Endometrial carcinomas are the most common type of uterine corpus malignancies, with sarcomas making up less than 10% of cases.Endometrioid carcinomas account for almost 83% of uterine corpus malignancies. Approximately 4% to 6% of endometrial carcinomas consist of more

aggressive types, such as virulent serous and papillary serous carcinomas. Clear cell carcinomas account for about 1% to 2% of cases [9]. It is crucial to distinguish between type 1 endometrioid and type 2 serous endometrial carcinomas, as well as other very aggressive non-endometrioid carcinoma histotypes. This distinction is necessary in order to comprehend, handle, and maybe prevent these diseases. The development of most endometrial endometrioid carcinomas starts with continuous growth of the endometrium, which is driven by either natural or artificial estrogen without any counteracting effect from progesterone or progestins. This growth progresses from simple to more complex types of endometrial hyperplasia (EH) [10]. While there have been numerous reports on omentum metastasis, there is a lack of research specifically examining omental micrometastases, especially isolated microscopic metastases in endometrial carcinoma (EC). Serum biomarkers have multiple applications in the detection, diagnosis, prediction of outcome, and monitoring of treatment for endometrial cancer micrometastasis. They play a crucial role in both primary and secondary prevention [11]. Trefoil family 3 (TFF3) is an oncogene that is regulated by estrogen. It belongs to the trefoil factor family, which consists of tiny proteins that are secreted and expressed by mucus secretory epithelia, primarily in the gastrointestinal system. There have been reports indicating that TFF-3 is excessively expressed in human neoplasms, such as intestinal, pancreatic, and prostate cancers. It has also been found to play a role in the advancement of gastric cancer. Furthermore, the TFF-3 gene in breast cancer is constantly controlled by estrogen, and similar associations have also been observed in endometrial cancer [12]. So, the aim of the present work was to study of the role of TFF3 as protein level for early detection of endometrial carcinoma micrometastases. Use blood sample instead of using invasive biopsies or expensive imaging techniques for detection of endometrial carcinoma micrometastases.

This case control study contained 50 patients and 25 age-matched healthy female volunteers as control group. Endometrial carcinoma patients were recruited from Obstetrics and gynecology department at Mansoura University hospital. The present study divided their participants into, Group 1 (control), Group IIA (non- metastatic EC) and Group IIB (metastatic EC), the mean age of group IIA (non- metastatic EC) was 61.92 ± 6.83years, Group IIB (metastatic EC) was 64.80 ± 7.67 and the mean age of control group (group 1) was 61.48 ± 7.98 years, with no statistically significant difference between them.While Alemdaroglu et al had examined a total of 397 patients diagnosed with EC Among these patients, 301 patients (75.8%) were <70 years old, while 96 patients (24.2%) were >70 years old. The median age was 63 (min: 33 and max: 89) in the entire group, 60 (min: 33 and max: 69) in the <70 age group, and 74 (min: 70 and max: 89) in the >70 age group, which in same line with our results [13]. The present study found a significant difference in weight between the three groups, group IIA and group IIB have significantly higher weight (93.24 ± 7.92), (97.56 ± 6.12) respectively compared to controls (75.76 ± 9.20). Most of the risk factors for developing endometrial cancer involve high levels of estrogens and obesity is associated with excessive levels of estrogen [14], indicating obesity increases the risk of developing endometrial cancer. In Gao et al, study, they found that the median BMI of women diagnosed with stage 1 endometrial cancer was significantly higher than in women diagnosed with stage II or III, with no difference in BMI between stage II and stage III cancer, suggesting that this negative relationship is restricted to early-stage endometrial cancer, that patients with higher BMI may have better clinical outcomes [15]. The present study revealed no significant difference between both groups regarding number of pregnancies and proportion of parous and nulliparous individuals. On other hand, many pregnancy-related factors are associated with reduced endometrial cancer risk. Trabert et al, had found that increasing number of pregnancies [≥four versus one birth and shorter time since last birth (<10 versus ≥30 years) were associated with reduced endometrial cancer risk, with consistent associations across most subtypes. Thus support the role for both hormonal exposures and cell clearance as well as immunologic/inflammatory etiologies for endometrial cancer [16]. The current study revealed slight difference in menopause stage distribution between the three groups, with a higher percentage of individuals in cases of group IIA and group IIB being in the Menopause stage compared to control group

(92.0%, 100%vs. 84.0%) respectively which was not statistically significant. In agreement with previous meta-analysis that examined Eighteen articles including 957242 subjects with 4781 cases. The pooled RR (95%CI) of endometrial cancer for the highest versus the lowest age at menopause was 1.89. For dose-response analysis, a nonlinear relationship was found between age at menopause and endometrial cancer, and the positive association became statistically significant when age at menopause was greater than 46.5 years old [17]. The current investigation demonstrated that Group IIA consisted of 25 patients, predominantly in Stage I and II, with 56% in Stage I and 44% in Stage II. All patients in Group IIA are not in Stage III or IV. In contrast, Group IIB, which also comprises 25 individuals, primarily falls into Stage III (52%) and Stage IV (48%), with no patients classified in Stage I or II.Lee et al. discovered that around 75% of patients with endometrial cancer are identified with early-stage illness (Stage I and II), whereas the remaining 25% are diagnosed with advanced-stage disease (Stage III and IV) [18]. When comparing primary tumor size in the present study, there was no significant difference between the two groups, with 64% of Group IIA and 52% of Group IIB having tumors smaller than 5 cm. Regarding metastasis in the current study, metastasis in this group is quite diverse, with the highest frequency of cases occurring in the lung, pelvic region, and peritoneum, each accounting for 28% of the cases. Meanwhile, bone metastasis is less common, comprising only 16% of the cases. These findings highlight the propensity of endometrial carcinoma to spread to various anatomical sites, with a preference for intrapelvic and intraperitoneal locations. Sohaib et al. conducted a review of 86 patients who had recurrent endometrial carcinoma after their initial surgery. They discovered that the disease recurred in different ways: locally in 30 patients (35%) with a median time to relapse of 11.5 months, distally in 32 patients (37%) with a median time to relapse of 20.5 months, and both locally and distally in 24 patients (28%) with a median time to relapse of 8.5 months. The sites where the cancer returned were lymph nodes in 41 (48%) patients, vagina in 36 (42%) patients, peritoneum in 23 (27%) patients, lung in 21 (24%) patients, hydronephrosis in 20 (23%) patients, bladder in 7 (8%) patients, liver in 6 (7%) patients, bone in 6 (7%) patients, abdominal wall in 6 (7%) patients, spleen in 4 (5%) patients, rectum in 3 (3%) patients, pancreas in 1 (1%) patient, muscle in 1 (1%) patient, and brain in 1 (1%) patient [19].TFF3 protein levels in the present study showed a marked disparity, with Group II having a substantially higher mean value (4.94 ± 3.47) compared to Group I (0.41 ± 0.15).These findings strongly suggest that TFF3 protein levels, is significantly elevated in endometrial carcinoma compared to the control group. Bignotti et al. discovered that in the majority of EC cases, TFF3 mRNA was consistently increased when compared to control tissues, as indicated by microarray data. [20]. This was consistent with our findings.Furthermore, Neubert et al conducted a study on 53 patients who had been diagnosed with endometrial cancer. Among these patients, six were diagnosed with endometrial hyperplasia at an average age of 62.9 ± 6.4 years (ranging from 55 to 74 years), while thirty patients diagnosed with endometrial atrophy at an average age of 63.3 ± 9.3 years (ranging from 48 to 62 years) were included as the control group. The study aimed to examine the levels of TFF1, TFF2, and TFF3 in these patients.The researchers discovered that levels of TFF3 were notably elevated in individuals diagnosed with endometrial cancer, but not in the subgroup of patients with endometrial hyperplasia. There were no significant differences in the levels of TFF1 and TFF2 across the identified histopathological subgroups [21]. Thus the current study suggested a potential association between elevated TFF3 levels and the metastatic progression of endometrial carcinoma. Group IIA, representing non-metastatic endometrial carcinoma, also demonstrates significantly higher expression levels compared to the control group (Group I). These findings indicate that TFF3 protein expression may serve as valuable biomarker for distinguishing between non-metastatic and metastatic forms of endometrial carcinoma. The present study revealed a remarkably high area under the ROC curve (AUC) values for a parameter, with an exceptionally high AUC values of 0.999 for TFF3 protein levels. These AUC values suggest that marker are excellent at distinguishing between healthy individuals and those with endometrial carcinoma.TFF3 protein levels has (98% sensitivity and 100% specificity). With high diagnostic potential of TFF3 protein levels in

identifying endometrial carcinoma, making them valuable candidates for clinical use in early detection and patient management.The current study found a positive correlation between TFF3 protein level and body weight. The age and number of pregnancies does not exhibit a significant correlation with TFF3 protein levels. Furthermore, the average serum levels of TFF3 in patients with CKD, metastatic and secondary carcinoma (MC), and acute gastroenteritis (AG) were considerably elevated compared to patients with other prevalent clinical conditions. Specifically, the mean concentrations were 200.9 ng/ml, 95.7 ng/ml, and 71.7 ng/ml, respectively.An inclination towards a positive association was noted between the levels of TFF3 in the blood serum and the severity of chronic kidney disease (CKD). The average serum TFF3 levels for stages 1–5 of chronic kidney disease were 23.6 ng/ml, 29.9 ng/ml, 54.9 ng/ml, 85.0 ng/ml, and 176.6 ng/ml, respectively.Similar patterns were noted in the levels of TFF3 in the urine and the stages of chronic kidney disease (CKD). Therefore, the expression of TFF3 was mostly localized in the tubular epithelial cells [22].In the univariable analysis conducted in this study, a positive correlation was shown between higher body weight and an elevated risk of endometrial cancer. The levels of TFF3 protein show a substantial correlation, indicating that higher expression levels are linked to a higher likelihood of developing endometrial cancer.In their meta-analysis, Jenabi and Poorolajal discovered that a total of 40 research were included, consisting of 20 prospective cohort studies and 20 case-control studies. These studies involved a combined total of 32,281,242 participants. The findings from both cohort and case-control studies demonstrated a substantial correlation. In comparison to individuals with a normal weight, the estimated relative risk (RR) of developing endometrial cancer was 1.34 for overweight individuals and 1.43 for obese individuals in the cohort study. In the case-control study, the estimated RR was 2.54 for overweight individuals and 3.3 for obese individuals. These results strongly suggest that there is a significant association between body mass index (BMI) and an elevated risk of endometrial cancer. Additional research is necessary to understand the underlying mechanisms of endometrial cancer resulting from overweight and obesity [23]. In the multivariable analysis, TFF3 protein levels, maintain their significance, findings highlight the potential utility of TFF3 protein levels as independent predictors of endometrial carcinoma susceptibility. Furthermore, there was a notable upregulation of TFF1 in gastric cancer. Additionally, the expression levels of both TFF1 and TFF3 were significantly elevated in 97 patients with colon cancer compared to 79 healthy individuals [24]. Researchers utilized an immune-histochemical technique to determine that the level of TFF3 expression in thyroid papillary carcinoma was greater than in the surrounding pre-carcinomatous tissue in 31 instances of thyroid papillary carcinoma[25].According to a clinical investigation, the rate of positive expression of TFF3 grew progressively from normal gastric tissues to paracancerous tissues and gastric cancer tissues. The rates were 5.0%, 40.0%, and 58.6% respectively.The presence of TFF3 in gastric cancer tissues was found to be associated with the extent of local lymph node metastases and the predicted outcome [26].A separate investigation revealed that the level of serum TFF3 in individuals with stomach cancer was notably elevated compared to those who were healthy [25].There was a direct association between the elevated levels of TFF3 protein in breast cancer and factors such as tumor size, lymph node metastasis, as well as unfavorable survival and prognosis [27]. Furthermore, a two-step immunohistochemical technique was employed to identify the presence of TFF3 in 90 sets of hepatocellular carcinomas (HCC) tumor tissues and neighboring non-cancerous tissues.The findings revealed that the rate of positive expression of TFF3 was 62.1% in tumor tissues and 33.8% in surrounding tissues.The distinction between the two groups was statistically significant [28].

6. CONCLUSION

In conclusion, the current study documented the critical role of TFF3 protein levels in understanding the molecular distinctions between non-metastatic and metastatic endometrial carcinoma.The findings hint at the possibility of using TFF3 protein levels to aid in diagnosis, prognosis, and treatment decisions for patients with endometrial carcinoma, especially for distinguishing between different stages of the disease.

7. REFERENCES

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