International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 5, 2025
https://theaspd.com/index.php

Beyond The Horizon: The Predictive Power Of FOXA2 And Microrna-590 Expression

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Abstract
This case-control study aimed to investigate the diagnostic and prognostic potential of FOXA2 and MicroRNA-590 (specifically its fold change) in ovarian cancer, correlating their expression levels with disease stage and grade. The study included 70 ovarian cancer patients and 35 healthy controls, conducted from October 2024 to September 2025 at from oncology unite in imam Al-Hussein Medical city & imam Al-Hassan Al-Mujtaba Hospital in Kerbala city. Sociodemographic, clinical, and biomarker data were collected via a structured questionnaire, and statistical analysis was performed using Graphical Pad Prism 9.
Demographic analysis of the patient cohort (N=70) revealed that the majority were aged 46-60 years (52.9%), obese (67.1%), housewives (88.5%), and resided in Kerbala (57.1%). A high proportion presented with Stage 4 disease (60%) and high-grade tumors (67.1%), with most receiving chemotherapy (88.5%) and exhibiting metastasis (77.1%).
Comparing biomarker levels between ovarian cancer patients and healthy controls, a statistically significant decrease was observed for both FOXA2 (patients: 3.09±1.98 vs. controls: 3.72±3.03, p=0.025) and MicroRNA-590 Fold Change (patients: 2606.50±1200.64 vs. controls: 4788.31±2223.37, P=0.009). Further analysis across disease stages indicated that MicroRNA-590 Fold Change was lowest in early-stage (Stage 1&2: 104±183) and increased in later stages (Stage 3&4: 3148±4451), though remaining significantly lower than controls (10085±12070). FOXA2 levels Were also Lower in patients Compared to controls, with a slight decrease from early (3.1±1.4) to late stages (2.9±1.1). Regarding tumor grade, FOXA2 Levels Were significantly lower in High-Grade Tumors (2.7±0.85) compared to low-grade tumors (3.3±1.5) and controls (5.2±3.9, p=0.003). MicroRNA-590 Fold Change was lowest in low-grade tumors (1340±955), higher in high-grade tumors (3451±1750), and highest in controls (10085±9070, P=0.05).
These Findings suggest That FOXA2 and MicroRNA-590 Fold Change are Significantly altered in Ovarian Cancer patients, correlating with disease Stage and Grade. This downregulation supports their potential as valuable diagnostic and prognostic biomarkers for Ovarian Cancer.
Keywords: *FOXA2, Fold Change, Ovarian Cancer, FIGO Stage*

1.INTRODUCTION

Ovarian Cancer (OC) the leading cause of Death among gynecological Cancers globally and is The eighth most frequently diagnosed malignancy , With over 314,000 New Cases and 207,000 deaths reported in 2020 alone (1). Despite advances in management and surgical techniques, most patients are Diagnosed at Late FIGO stages (III–IV), leading to a 5-year survival rate below 50% (2). However, The Absence of Effective Screening methods and The non-specific nature of Early symptoms pose significant diagnostic challenges (3). Epithelial Ovarian Cancer (EOC), accounting for 90% of OC cases, is a Heterogeneous Disease Composed of distinct Histological and molecular Subtypes, including High-Grade Serous (HGSC), clear cell, endometrioid, and mucinous carcinomas (4,5). The FIGO staging system remains the gold standard for classifying disease extent, while the histological grading system offers insight into tumor aggressiveness and prognosis (6). Nevertheless, grading remains largely subjective and lacks molecular precision (7), emphasizing

the need for more reliable biomarkers. Current diagnostic biomarkers, such as CA-125 and HE4, have Limitations in Sensitivity and Specificity, Especially in Early Stages or among specific histologic subtypes (8,9). Thus, research has shifted toward exploring molecular signatures like microRNAs (miRNAs) and transcription factors for their diagnostic and prognostic potential (10). MicroRNA-590 (miR-590), a non-coding RNA located on chromosome 7q11.23, Plays a critical role in regulating Cell Proliferation, Differentiation, and tumor progression (11). Its dysregulation has been implicated in Various Cancers, including cervical, breast, and hepatocellular carcinoma, often acting as an oncogene (12). In ovarian cancer, studies suggest that miR-590 may promote tumor growth by targeting tumor suppressor genes, including cyclin regulators like cyclin G2 (13). Forkhead box protien A2 (FOXA2), a member of the FOX transcription factor family, functions in embryonic development and cellular metabolism (14). Recent studies highlight FOXA2 as a Tumor Suppressor in Several Cancers Such As pancreatic, lung, and gastric Cancer (15). There is growing evidence of a regulatory interplay where miR-590 suppresses FOXA2, potentially facilitating oncogenesis (16). This Study aims To assess the Association between These biomarkers and Disease classification, exploring their utility in improving diagnostic precision beyond histopathology.

2 MATERIALS AND METHODS

2.1. Study Design and Subjects: Between January 2024 and August 2025, 70 Ovarian Cancer Patients and 35 Healthy Controls Were selected from oncology unite in Imam Al-Hussein Medical City and Imam Al-Hassan Al-Mujtaba Hospital in Kerbala For this case-control study, which involved 105 blood samples. Participants were categorized according to:

- FIGO stage: Stage I–II (Early) and Stage III–IV (Late)
- Tumor grade: (Low grade) and (High grade)

Control samples were collected from Age-Matched Healthy Individuals with no History of Cancer or autoimmune disease.

2.2. Ethical Approval: Informed Consent Was Obtained from all participants. Ethical Clearance Was granted by The Ethical Committee of Kerbala Medical College and hospital administration.

2.3. Sample Collection and Storage: A 5 mL of peripheral Blood Was Drawn from Each Participant. Samples were collected in el tubes for serum separation (used for FOXA2 measurement) EDTA tubes for RNA extraction (used for miR-590 analysis) serum Was Separated by Centrifugation at 4000 ×g For 10 minutes and Stored at −80°C until Analysis.

2.4. *Measurement of biomarkers*: A human FOXA2 sandwich ELISA kit (BT Lab, China; Cat. No. E7843Hu) with a Sensitivity of 0.1 ng/mL and an Assay Range of 0.19–12 ng/mL was used to assess the serum FOXA2 concentration. At 450 nm, absorbance was measured, and concentrations were computed using the standard curve. The Total RNA Mini Kit (Genaid, Taiwan) Was Used to extract total RNA from whole blood for fold change analysis, and qRT-PCR was used to measure the levels of gene expression. the 2^−ΔΔCt technique Was used to Calculate Fold Change in order To evaluate the relative expression levels among various patient groupings.

2.5. Group Classification for Biomarker Comparison: Participants were grouped as follows to assess biomarker variation:

- By Stage (FIGO):
- Group A: Stage I–II (early stage)
- Group B: Stage III–IV (late stage)
- By Tumor Grade:
- (high grade)
- (low grade)

2.6. Statistical Analysis: Statistical Analyses Were Conducted Using SPSS v28.0 and Real Statistics Excel Pack. Differences in FOXA2 and miR-590 fold change were analyzed using t-tests and ANOVA Association with stage and grade was evaluated. Statistical Significance Was set at $P < 0.05$.

3.RESULT

table 1 presented The descriptive Demographic Characteristics of The Study population. The Majority of The Study Participants (52.9%) Were in the Age Group of 46-60 Years. The 30-45 years age group comprised 22.9% of the population, while the 61-75 years age group accounted for 24.2% .

Results were shown that about (67.1%) of the study population were classified as obese. Overweight individuals constituted 24.1% of the sample, and only 8.5% were within the normal weight range. The prevalence of a positive family history of the disease was slightly lower than a negative family history. 52.8% of the participants reported a family history of the disease, while 47.1% reported no family history.

The predominant educational level among the participants was "Housewife," representing 88.5% of the sample. Employees constituted 7.1%, retired individuals 2.8%, and students 4.0 %.

The majority of the participants resided in Kerbala (57.1%, followed by Babil (31.4%. Smaller proportions resided in Bagdad (4.2%), Nasiriyah (2.8%), and Diyala (4.2%).

The most common disease stage observed in the study population was Stage 4, accounting for 60% of the participants. Stage 1 was observed in 14.2%, Stage 3 in 15.7%, and Stage 2 in 10%. A high grade of the disease was present in 67.1% of the study participants, while a low grade was observed in 30%.

Table 1: Descriptive of The Demographic Characteristics of The Study population (N=70).

Variable	Groups	N	%
Age. Groups	30-45 Years	16	22.9
	46-60 Years	37	52.9
	61-75 Years	17	24.2
BMI.groups	Normal weight	6	8.5
	Over weight	17	24.2
	Obesity	47	67.1
family history of disease	Yes	33	47.1
	No	37	52.8
educational level	Employees	5	7.1
	Housewife	62	88.5
	Retired	2	2.8
	Student	1	4.0
residence	Kerbala	40	57.1
	Babil	22	31.4
	Bagdad	3	4.2
	Naserha	2	2.8
	Diyala	3	4.2
Stage	Stage 1	10	14.2
	Stage 2	7	10
	Stage 3	11	15.7
	Stage 4	42	60
Grade	High	47	67.1
	Low	21	30

	Others	2	2.8
chemotherapy	Yes	62	88.5
	No	8	11.4
Metastasis	Yes	54	77.1
	No	16	22.8

Table 2 demonstrated the mean differences of the selected biomarkers (FOXA2, MicroRNA-590, and Fold change) and compared their levels Between The Ovarian Cancer patient group and The Control Group. The mean Level of FOXA2 in ovarian cancer patients was 3.09. in The Control Group, The mean FOXA2 level was 3.72. A T-test revealed a statistically significant difference Between The Groups (P = 0.025).
On The other hand, the Fold change value of MicroRNA-590 in ovarian cancer patients was 2606.50, and in the Control, Group was 4788.31. The T-test indicated a statistically significant difference Between these means (P = 0.009).

Table 2: Mean differences of biomarkers between Ovarian cancer patients & Control Groups

Biomarker	Patients N=70	Control N=35	P value
FOXA2	3.09±1.98	3.72±3.03	0.025[S]
Fold change	2606.50±1200.64	4788.31±2223.37	0.009[S]
T test was *: Significant at p ≤ 0.05 N: number of cases; SD: Standard Deviation; S: significant; NS= Non Significant			

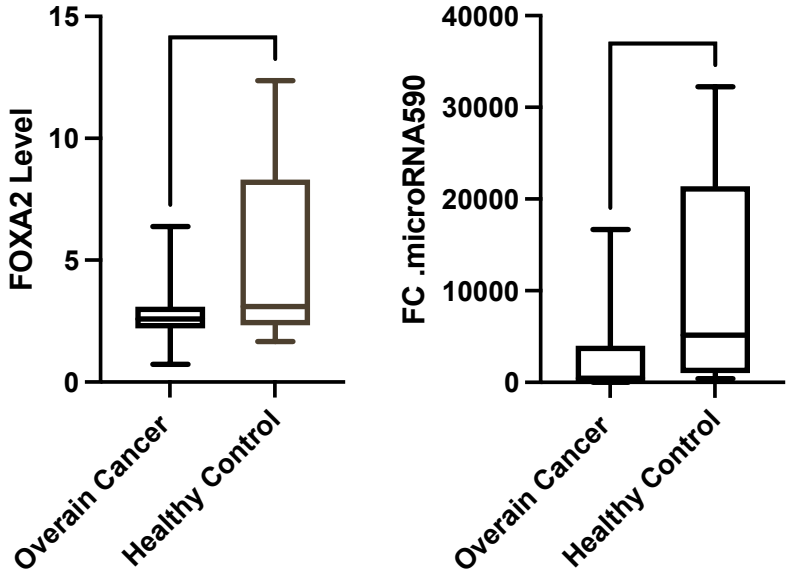

Figure 1: Results of The analysis of basic *Ovarian Cancer* for patients with Control Groups (T-test was S= Significant at p ≤ 0.05, NS= Non-Significant). *(t- test was *: Significant at p ≤ 0.05, **: significant at P ≤ 0.01, *: Significant at P ≤ 0.001, ****: Significant at P ≤ 0.0001)***

Table 3 presents the mean differences of two selected biomarkers, FOXA2 and Fold change, among different stages of ovarian cancer patients. The mean and Standard Deviation (±SD) for each biomarker Were shown. The P-value from a Statistical Test comparing The means across the stages was also reported for each biomarker.

The alteration was most dramatic for Fold Change, which is very low in early-stage cancer, Fold change values were notably different across the groups. The healthy control group exhibits the highest fold change (10085 ± 12070), followed by the ovarian cancer Stage 3 & 4 group (3148 ± 4451). The ovarian cancer Stage 1 & 2 group shows the lowest fold change (104 ± 183). This indicated that fold change is substantially Reduced in Ovarian Cancer patients, and this reduction is more pronounced in earlier stages of The disease.

FOXA2 levels also vary, levels Were lower in Ovarian Cancer patients than in Healthy Controls, with only a slight difference between early and late-stage disease. The healthy control group has the highest FOXA2 levels (5.2 ± 3.9). Ovarian cancer patients in both Stage 1 & 2 (3.1 ± 1.4) and Stage 3 & 4 (2.9 ± 1.1) show lower levels of FOXA2 compared to the healthy controls. There is a slight decrease in FOXA2 from earlier to later stages of ovarian cancer, but the difference between the two stages is small.

Table 3: Mean difference of Biomarkers among different Stages of Ovarian cancer patients compared to their FIGO stages and control group

	Ovarian cancer Stage 1&2	***Ovarian cancer*** Stage 3&4	Healthy Control
Fold change	104±183	3148±4451	10085±12070
FOXA2	3.1±1.4	2.9±1.1	5.2±3.9

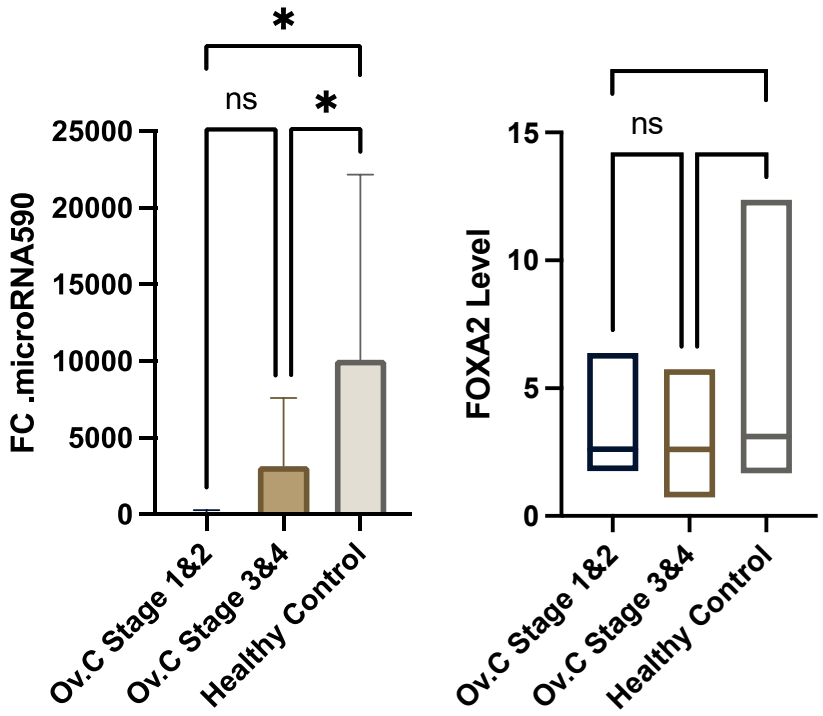

Figure 2: Distribution of Biomarkers among different Stages of Ovarian Cancer patients compared to their FIGO stages and Control group, *(Post Hoc ANOVA test was *: Significant at P ≤ 0.05, **: significant at P ≤ 0.01, *: significant at P ≤ 0.001, ****: significant at P ≤ 0.0001)***

Table 4 presents The mean differences of two selected biomarkers, FOXA2 and Fold change, Between The High grade and Low grade groups of Ovarian Cancer patients. The mean and Standard Deviation (±SD) for each biomarker Were provided for both grades, along with the p-value from a T-test comparing the means between the two groups. Both FOXA2 and Fold Change show statistically significant differences between the groups, indicating that these biomarkers are altered in Ovarian Cancer patients Compared to Healthy individuals. There is a general Trend for both FOXA2 and Fold Change to decrease as The grade of Ovarian Cancer increases, although The difference between high Grade and low Grade is not as pronounced as the difference between either of the Grades and the healthy controls.

FOXA2 levels were significantly different across the groups (P value = 0.003). Specifically, FOXA2 levels are highest in the healthy control group, followed by low Grade ovarian cancer, and are lowest in high Grade ovarian cancer.

Fold change values also shown significant differences across the groups (P value = 0.05). The healthy control group has the highest fold change, followed by High Grade , with Low Grade Ovarian Cancer showing the lowest fold change.

Table 4: Mean difference of Biomarkers among different grades of *Ovarian cancer* patients & Healthy *control group*

Biomarkers	High Grade	Low Grade	Healthy Control	**P value**
FOXA2	2.98±0.85	3.2± 1.5	5.2±3.9	0.003[S]
Fold change	3451±1750	1340±955	10085±9070	0.05[S]

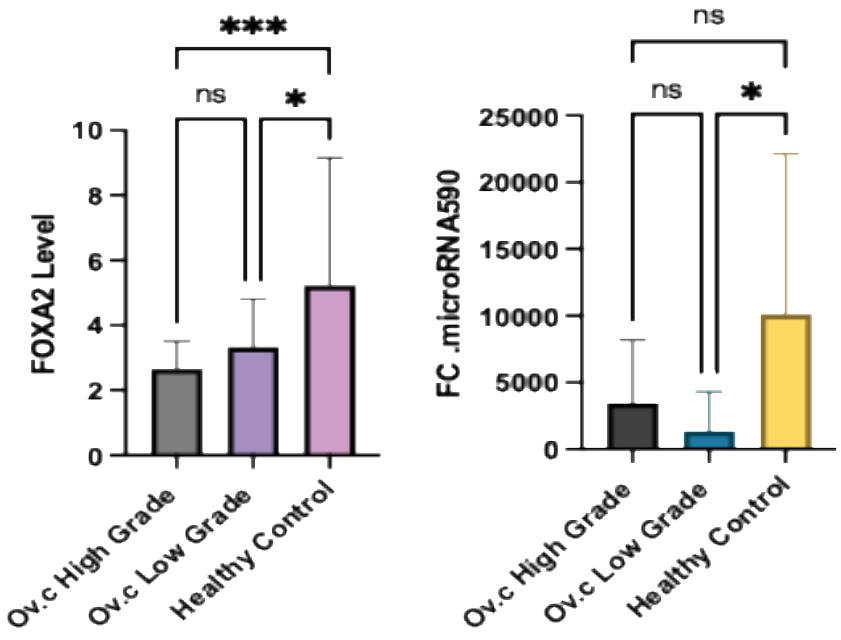

Figure 3: Distribution of Biomarkers among different grades of Ovarian cancer patients and control group *(Post Hoc ANOVA test was *: Significant at P ≤ 0.05, **: Significant at P ≤ 0.01, *: Significant at P ≤ 0.001, ****: Significant at P ≤ 0.0001)***

DISCUSSION

The intention of the current Study Was to determine how The Expression levels of FOXA2 and Fold Change in ovarian cancer patients related to the disease's grade and stage. The results revealed a significant decrease in FOXA2 expression in Ovarian Cancer patients Compared to healthy controls. This Finding Supports previous Studies that have indicated the downregulation of FOXA2 in Various Types of Cancer, including Ovarian Cancer (17). FOXA2 is known to Play a Crucial role in Regulating gene expression during embryonic Development and maintaining cellular homeostasis (14). Its reduced expression in cancerous tissues has been associated with increased tumor aggressiveness and poor prognosis (18). The observed decrease in FOXA2 expression in Ovarian Cancer patients in this study further corroborates these findings and suggests that FOXA2 may Serve as a potential biomarker For disease progression and prognosis.

Regarding the fold change values, the study demonstrated a Significant reduction in Ovarian Cancer patients compared to the control group. Fold change analysis is a commonly used Method to assess The Relative expression levels of Target genes (19). The downregulation observed in this study aligns with previous research indicating altered gene expression patterns in cancer patients. Notably, the fold change values were lower in early-stage ovarian cancer patients and gradually increased in advanced stages, although they remained Significantly lower Than Those in The Control Group. This trend suggests that changes in gene expression occur early in the disease and may continue to evolve as the disease progresses (13).

The analysis of biomarker expression based on tumor grade further supported these findings. Both FOXA2 and Fold Change levels Were Significantly lower in High-Grade tumors compared To Low-Grade Tumors. This observation is consistent with the hypothesis that higher-grade tumors exhibit more aggressive behavior and greater molecular alterations (13). The significant differences in biomarker levels between different grades underscore the potential utility of FOXA2 and Fold Change as indicators of tumor differentiation and aggressiveness.

Overall, The Results of This Study Contribute to the Growing Body of evidence Supporting The role of FOXA2 and gene expression changes in The pathogenesis of Ovarian Cancer. Furthermore, Zhang et al. (2016) (20) demonstrated that FOXA2 overexpression independently predicted reduced lymph node metastasis, indicating its role as a favorable prognostic marker in Ovarian Cancer. The observed associations between biomarker levels and disease stage and Grade highlight their potential as diagnostic and prognostic tools. Further Research is needed To validate These findings and explore The underlying Mechanisms regulating FOXA2 expression and gene expression changes in Ovarian Cancer (16).

CONCLUSION

According to this study, individuals with ovarian cancer had considerably lower levels of both FOXA2 and Fold Change, which were also connected with the stage and grade of the disease. These results provide support to their possible application as prognostic and diagnostic biomarkers, which could help evaluate the course of a disease.

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