Peer Review File

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Reviewer A

Comment 1: Figure 2 only shows a total of 39 patients from the 100 patient cohort. What is the criteria for this selection? Legends need to specify this.

Reply 1: We thank the reviewer for this comment. Figure 2 represents the overall survival for the whole protein cohort. The total of 39 patients was based on the cut-off that we used in our analysis. We used the median (H-score = 140) as the cut-off for low and high FXR1 expression, as indicated in the text. Furthermore, we were unable to obtain survival data for some of the 100 patients because they did not follow up with the hospital from which we gathered the data.

Change in the text: While our study's results are remarkable, it had some limitations. One of these limitations is the small number of clinical samples. Furthermore, the hospital where we gathered the data did not follow up with some of the 100 patients, making the survival data unavailable. However, the data provided high statistical power and enabled us to identify a novel biomarker associated with aggressive behavior in BC. (see Page 11, lines 248-251)

We have updated Figure 2 and added the legend to clarify that. (See Figure 2)

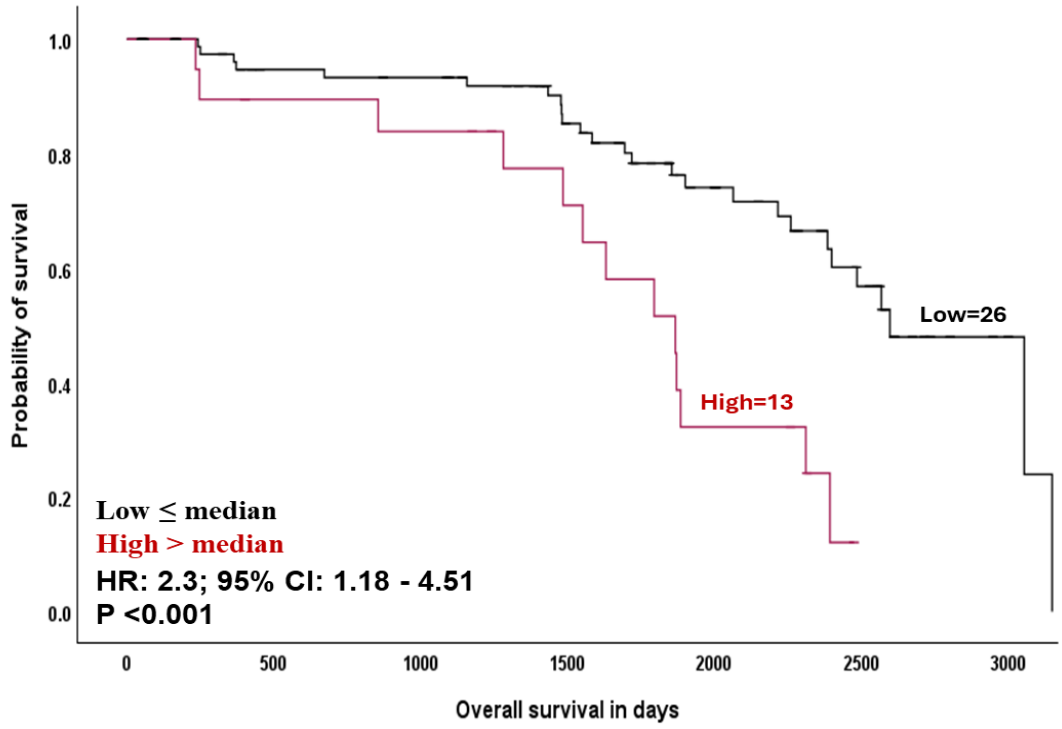


Figure 2. Kaplan–Meier survival plots showing the association between FXR1 protein expression and overall survival in KASH cohort

Comment 2: It is not clear why FXR1 high TNBCs have better outcome according to the authors even though their KM plot shows overall poorer outcome. I would like to see two additional KM plots, one for TNBCs and one for all non-TNBC. If the numbers are not statistically significant, I would still include as a supplementary figure given this difference.

Reply 2: We thank the reviewer for this comment. High FXR1 was associated with poorer outcomes in the whole cohort. However, the analysis of TNBCs and all non-TNBC shows better outcomes even though the KM plot shows an overall poorer outcome in the whole cohort. To validate that, we utilized publicly available datasets (bc-GenExMiner) version 5 (https://bcgenex.centregauducheau.fr) as a prognostic analytical module. The results confirmed our findings that high FXR1 was associated with better outcomes in BC molecular subtypes. The findings suggest that FXR1 has the potential to serve as a prognostic biomarker in BC. It is intriguing that our analysis of the entire cohort revealed that a high FXR1 level was associated with a poor outcome but not in a specific molecular subtype. Therefore, categorizing patients according to their molecular subtype appears to invalidate the prognostic value of FXR1. This phenomenon continues to be ambiguous and necessitates additional clinical research to be approved. The publicly accessible data that were used in this study have verified the prognostic value of FXR1.

Change in the text: **Results section:** ***Association of FXR1 protein levels with patient outcomes:*** In the univariate analysis, a high FXR1 protein level was associated with shorter OS ($p < 0.001$; Fig. 2). In the Cox regression analysis of the KASH cohort, a high FXR1 protein level was a significant predictor of shorter OS regardless of lymph node status, tumor size, and tumor grade (hazard ratio = 3.079, 95% confidence interval = 1.055–8.986, $p = 0.040$; Table III). However, no statistical significance was found when the data was categorized into TNBCs and all non-TNBC (Supplementary Figure 3). (see Page 9, lines 181-182)

FXR1 mRNA levels

In order to validate our protein-level results, *FXR1* mRNA levels were determined 149 in all public DNA microarray datasets in the BC Gene Expression Miner database (version 5.0; $n = 10,872$). An exhaustive expression analysis found that *FXR1* mRNA levels were significantly higher in patients who were younger (aged <51 years) or had basal-like or TNBC (all $p < 0.0001$). High *FXR1* mRNA levels were also associated with the receptor statuses ER− ($p < 0.0001$), PR− ($p < 0.0001$), and HER− ($p = 0.004$; Figure 3). No significant correlations were observed with the other clinicopathological parameters.

Additionally, bc-GenExMiner version 5 (https://bcgenex.centregauducheau.fr), a publicly available dataset, was used as a prognostic analytical module to validate the prognostic significance of FXR1. The results confirmed our findings that high FXR1 was associated with poor prognosis in the whole cohort. However, there is no statistical significance in any BC

molecular subtypes (Supplementary Figure 4).(see Page 9, lines 189-193)

Discussion section: The findings of our study suggest that FXR1 has the potential to serve as a prognostic biomarker in BC; however, it is intriguing that our analysis of the entire cohort revealed that a high FXR1 level was associated with a poor outcome but not in a specific molecular subtype. Therefore, categorizing patients according to their molecular subtype appears to invalidate the prognostic value of FXR1. This phenomenon remains questionable and necessitates additional clinical research to be approved. Thus, the publicly accessible data that were used in this study have verified the prognostic value of FXR1. (see page 10, lines 224-230)

Comment 3: "Overall, all these results indicate that FXR1 might act as a tumor promoter in BC" The data on PCR and proliferation does not support this statement by the authors. Either revise or explain this discrepancy.

Reply 3: We thanks the reviewer's comment. We have revised the text as suggested.

Changes in the text: Interestingly, in our study, higher FXR1 protein levels were associated with low Ki-67. The low level of Ki-67 in these patients may be due to the neoadjuvant chemotherapy they may have received (22). A previous study supports this by demonstrating a significant association between elevated FXR1 expression and a pathological complete response (pCR), which is characterised by the absence of residual invasive and in situ carcinoma on hematoxylin and eosin assessment of the entirely excised breast specimen and all examined regional lymph nodes after the completion of neoadjuvant therapy. Therefore, FXR1 may be considered an independent predictive biomarker for better response to neoadjuvant chemotherapy in patients with high FXR1 levels (9). However, FXR1 protein levels warrant further analysis in the context of chemotherapy responses and care. (see Page 10, lines 215-223)

Comment 4: The 351.9% increase in BC rates in the abstract seems large and the citation for this data does not specific that number. What is this based on?

Reply 4: We appreciate the reviewer's comment. Since 2001, the number of breast cancer diagnoses among Saudi women has more than tripled, rising from 545 cases to 2463 cases in 2017. We have presented this information in a straightforward manner, using only percentages. However, we have changed this percentage in words instead of the percentage to avoid any confusion in both the abstract and the text. We have also added more proper citation (a 17-year retrospective analysis for BC incidence in Saudi female population) (see Page 3, lines 48-49; and see Page 9, lines 195-196).

Changes in the text: **Abstract section** " The number of BC cases in Saudi Arabia has more than tripled during the last 17 years to constitute 30% of all cancer cases in women (see Page 3, lines 48-49)"

Discussion section " In Saudi Arabia, BC is the leading cancer in women as the number of BC cases in Saudi women has more than tripled during the last 17 years (see Page 9, lines 195-196) ".

We also have changed the reference cited to support this information (see Page 13, line 283)

Comment 5: At minimum, I would like to see 10 FXR1 high and 10 FXR1 low patient images as it is not clear how the single images shown are representative of the whole cohort. These can be supplementary images and ideally across the different subtypes.

Reply 5: We appreciate the reviewer's comment. We included a single image for each high and low expression of FXR1 to illustrate the differences in pattern between the low and high expressions (see Page 12, line 289). However, we have added 10 cases for each high and low FXR1 expression as requested.

Changes in the text: We have added 10 cases for each high and low FXR1 expression as requested. (see supplementary Figures 1 and 2).

Minor points

•"It is a 40 highly heterogeneous disease with 28 distinct histological subtypes " - Need citation for this.

Replay: We thank the reviewers for this comment. We have added the citation as requested (see Page 4, lines 72)

Line 45 needs citation

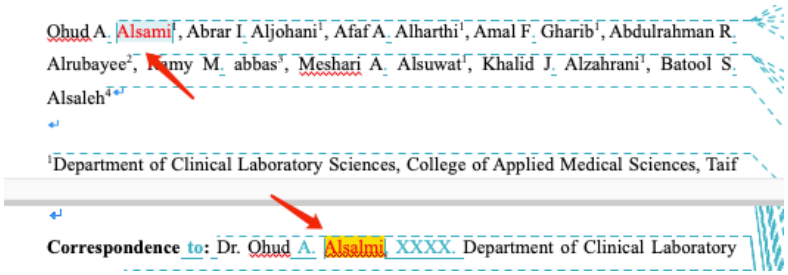
Replay: We thank the reviewers for this comment. The content has 3 sentences which were an explanation of the main information from citation number 5. Therefore, we only wrote the citation at the end of the third sentences. However, we have repeated the citation at the end of each sentence (see Page 4, lines 77-81).

•Line 47 needs citation

Replay: We thank the reviewers for this comment. The content has 3 sentences which were an explanation of the main information from citation number 5. Therefore, we only wrote the citation at the end of the third sentences. However, we have repeated the citation at the end of each sentence (see Page 4, lines 77-81).

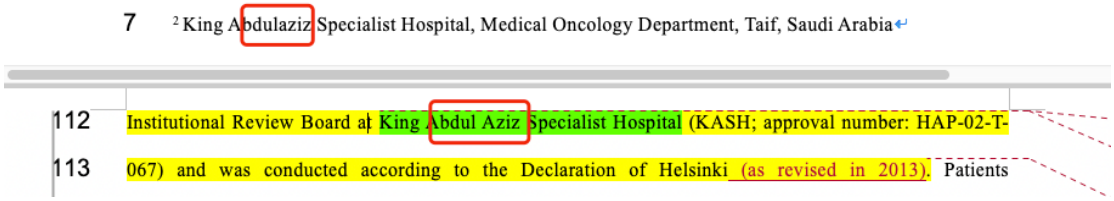
Reviewer B

1. The author name is not consistent in the title page, please check and unify.



We apologize for this typo. As requested, we unified the author's family name (see page 1).

2. Please unify the name of the unit throughout your whole text.



We apologize for this typo. As requested, we unified the name of the unit throughout the whole text (highlighted in yellow).

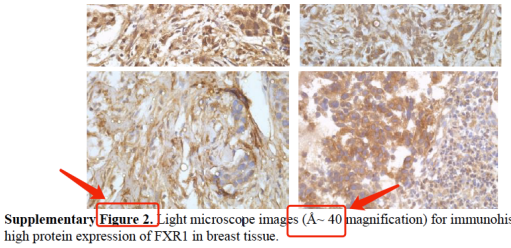
3. And Figure 3 is too vague to be published, please resend a **higher resolution** version.

We have sent Figure 3 in a high resolution (see Figure 3).

4. Please define ALL abbreviations in figure legends, including supplementary figure legends.

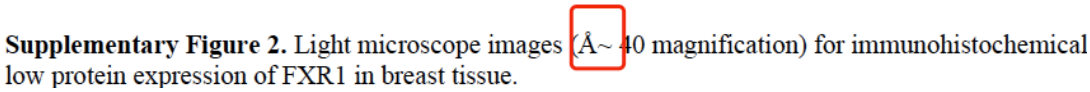
We have defined all abbreviations in figure legends as suggested (see pages15-18).

5. Should this be Supplementary Figure 1 for your paper? Please check the information marked in red below, it's confusing.



We apologize for this typo. We have checked and revised (see Supplementary Figure 1).

6. Same matter in Figure S2.



We apologize for this typo. We have checked and revised (see Supplementary Figure 2).

7. Please define ALL abbreviations shown in Table 1-3 in their table footnotes separately.

We have defined all abbreviations in Table 1-3 as suggested (see pages16).

8. Some data (including **percentages**) show inaccuracy in Table 1, please check and revise.

We apologize for this typo. We have checked all the percentages and revised them as requested (see Table 1).