

Review report

Final title: Expression of BRCA1 mRNA in cancerous and non-cancerous breast tissue of Bangladeshi females attending a tertiary care hospital

Title at submission: Expression of BRCA1 mRNA in cancerous and non-cancerous breast tissue of Bangladeshi females attending a tertiary care hospital

Submission date: 9-Oct-24
Revised submission: 24-Jan-25
Accepted: 5-Feb-25

ROUND 1

Reviewer F: Md. Fazlul Karim, ORCID: 0009-0008-5065-1887, COI: None

Overview This study examines BRCA1 mRNA expression in breast cancer and non-cancerous breast tissues among Bangladeshi females. It reports reduced or absent BRCA1 mRNA expression in both groups, with notable similarities between breast cancer and fibroadenoma tissues. The work suggests the inclusion of fibroadenoma cases in screening programs and highlights the need for molecular profiling. The manuscript is well-structured, but the sample size and limited scope of non-cancerous cases restrict the generalizability of the findings.	
1. Comment	The study provides significant insights into BRCA1 expression in an underrepresented population. However, the authors could elaborate on the potential implications of consanguinity in BRCA1 expression and further discuss the limitations regarding the small and heterogeneous non-cancerous sample.
Response	Consanguinity in BRCA1 expression is discussed in lines 250 to 272 and seven references are added. Limitations regarding the small and heterogeneous non-cancerous sample are explained in limitation lines 280-288.

Reviewer G: Md. Jahirul Islam, ORCID: 0000-0002-8825-1319, COI: None

Overview This study looks at BRCA1 mRNA expression in breast cancer and benign breast conditions in Bangladeshi women. Researchers studied tissue samples from 50 breast cancer patients and 19 women with benign lesions. They used RNA extraction, cDNA synthesis, and real-time RT-PCR to measure gene expression. The results show that BRCA1 expression is different in cancerous and non-cancerous tissues. These findings could help improve treatment and predict outcomes for patients. The research is well done and fills a gap in breast cancer studies in Bangladesh. It provides useful information for better understanding these conditions in this population.	
2. Comment	Are the table(s) and figure(s) clear and appropriate to address the objective(s)? = No In Table 1 the <i>P</i> value for "Cancerous tissue versus Non-cancerous tissue" is listed as 0.49, which shows no significant difference between the two groups. However, the <i>p</i> -values for individual non-cancerous tissue types (fibroadenoma, fibrocystic disease, etc.) are also listed, but their statistical context is unclear. Are these values comparing the tissue types to each other, or to cancerous tissue? It should be clarified. In Table 2 & 3 the name of the actual tests were not mentioned (whether Chi-squared test of Fisher's Exact test?)
Response	Table 1 is modified and comparison of BRCA1 mRNA expression was done between cancerous and non-cancerous breast tissue by Mann-Whitney U test. No comparison was done between various types of non-cancerous tissue. Figure 2 and Table 1 are modified. In Figure 2, median (range) of BRCA1 mRNA values, <i>p</i> -value and name of the test were added. In Table 1, the 2nd and 3rd columns were deleted. Tests are mentioned specifically in the foot note of Table 2 and 3.
3. Comment	The authors mention that abnormal methylation might affect BRCA1 expression, but it would have been helpful to explain more about how this methylation or LOH happens. The discussion talks about fibroadenoma, but it doesn't give much information about other non-cancerous conditions that were also studied.
Response	Methylation explained in lines 236-239, LOH explained in lines 233-235, information about other non-cancerous conditions that were also studied-added and explained in lines 215-224.

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4. **Comment** Use UK spelling throughout the manuscript. Check typos and grammar a little carefully.
Response UK English was used.
5. **Comment** Abstract: Cross-sectional study is enough. There is no need to write an analytical study.
Response Analytical study was removed.
6. **Comment** Highlights: Please replace it with a key message using 50-60 words in descriptive terms. This is because of the Journal's change in style.
Response A paragraph of 57 words is written.
7. **Comment** Sampling methods: How many eligible subjects attended NICRH between Jan 2021 to Sep 2022? This is necessary to know about the representativeness of the 62 patients with breast cancer and 27 women without breast cancer. If all eligible subjects were targeted and invited to participate, or a subsample of the eligible subjects were invited using an appropriate statistical approach, the study could be considered cross-sectional. Your study subjects' representativeness has to be clarified to claim your findings are valid.
Response List of total 915 breast cancer patients and 126 females with non-cancerous benign breast lesions were found in the register of the Department of Histopathology from January 2021 to September 2022. We included available all (68) FFPE blocks of non-cancerous breast tissue, 27 of which had sufficient amount of breast tissue and ultimately genetic analysis was possible from 19 samples. For selection of tissue blocks we emphasised on recently prepare FFPE sample, as quality of RNA is reduced with time. Lines 123 to 127 are modified for this in Method section.
8. **Comment** Add a subsection on ethical concerns to the Methods section you have addressed in this study. You have some of these points in the footnote (ethical approval). Keep the approval there, but write about the ethical concerns in the Methods section.
Response A subsection of ethical concern is added in the Method section in lines 171–178.
9. **Comment** Discussion: Summarise the first four lines to avoid duplication of results. Do not cite the tables and repeat the findings (lines 199, 222, 224).
Response Summarisation of the first four lines are done and duplication is avoided in lines 204-205). The citation of tables are removed and the repetition of findings is removed lines 242-244.
10. **Comment** Acknowledgements: Remove the funding statement from here but add these to the Funding subsection.
Response Funding statement is removed from the Acknowledgements, and added in the Funding subsection.
11. **Comment** List all authors (avoid *et al.*)
Response All the *et al.* is avoided in the Reference Section. Name of all authors are listed in all references.
12. **Comment** Tables: Results up to one decimal point should suffice for all tables. Table 2: You have 16 and 34 subjects to compare. Creating so many categories for such a small number of subjects. I recommend reporting quantitative data, such as age, BMI, age at menarche, age at menopause, number of children, and duration of contraceptive use. This will bring more analytic powers.
Response One point after decimal was added in case of percentage, statistical value was kept as it was. BRCA1 mRNA $2^{\Delta\Delta Ct}$ value was kept up to two decimals.
13. **Comment** Figure: The figure is a screenshot from the lab's computer. Please submit the original figure with a higher resolution.
Response Original Figure higher resolution is submitted.

ROUND 2

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14. **Comment** Author responses are published as a Review report. Therefore, a formal response is necessary. Responses like "added" or "done" are not publishable. Kindly write a complete response: what has been added or done.
Response A complete response has been written in every response.
15. **Comment** Figure 2 (line 165) presents the results; it should not be in the Methods section. Moreover, it is redundant to Table 1 (second column along with its *P* value, median expressions). I recommend you keeping the figure. It would be excellent if you could add the *P* value to the graph.
Response Figure 2 is referred in Results section (line 185-187) and removed from Methods section. (BRCA1 mRNA expression level ($2^{\Delta\Delta Ct}$ value) was not significantly different in the cancerous and non-cancerous breast tissue (FIGURE 2).
Figure 2 and Table 1 are modified. In Figure 2, median (range) of BRCA1 mRNA values, *P* value and name of the test were added. In Table 1, the 2nd and 3rd columns were deleted.