Author’s response to reviews

Title: BRCA Mutations in a Cohort of Iraqi Patients Presenting to a Tertiary Referral Center

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Author’s response to reviews:

Dear Editor

Thank you for considering our manuscript for publication “BRCA Mutations in a Cohort of Iraqi Patients Presenting to a Tertiary Referral Center”

Please find our responses to the editorial and reviewer’s comments below:

Comments:

1. DESCRIBE THE METHODS IN DETAIL MENTIONING CLEARLY THE SOURCE OF DNA.

   • Thank you, the methods have been elaborated in the text. The source of DNA from blood samples is mentioned.

2. IF SEQUENCING HAS BEEN DONE, MENTION THE PRIMER SETS FOR EACH EXON.

   • Thank you, this has been elaborated in the methods
3. PROVIDE THE ELECTROPHEROGRAMS.

• Thank you, these have been added

4. SEPARATELY MENTION THE MUTATIONS FOUND IN EACH TYPE OF CANCER AND INTERPRET THE RESULTS ACCORDINGLY.

• Unfortunately due to the retrospective nature of the study, further clinical information is not available for all patients since many were not treated at our institution and were referred specifically for BRCA testing in our referral center

5. ADD TABLE FOR CANCER TYPE AND STAGES

• Unfortunately due to the retrospective nature of the study, further clinical information is not available for all patients since many were not treated at our institution and were referred specifically for BRCA testing in our referral center

6. MODIFY DISCUSSION TO CORRELATE THE RESULTS OF PRESENT STUDY WITH ALREADY PUBLISHED LITERATURE ON THE TOPIC.

• Thank you, this is the first report of BRCA mutations found in patients from Iraq, we have discussed the literature on the spectrum of BRCA mutations found in the Middle East in general

7. USE A UNIFORM PATTERN FOR CITING REFERENCES IN THE MANUSCRIPT.

• Thank you, this has been updated

Reviewer reports:

Kamlesh Guleria (Reviewer 1):

1. In the introduction add information about BRCA1 and BRCA2 variants reported in Iraqi population.
• Thank you, this is the first report of BRCA mutations found in patients from Iraq, we have discussed the literature on the spectrum of BRCA mutations found in the Middle East in general. This has been clarified in the introduction.

2. In methods add how much blood sample was collect from each participant. Modify the lines 55 - 59 as "BRCA1 and BRCA2 genes were amplified using primers (Add list of primers used as supplementary Table)). Amplified PCR products were checked on 2% agarose gel. The amplicons were purified (Add purification method) and sequenced on Genetic Analyzer (Add model of instrument used) using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA).

• Thank you, we have amended the methods section with as much information as we have available.

3. Line 3-4 on page 4 "Modify this as reference sequences (BRCA1: RefSeq NM_007294; BRCA2: RefSeq NM_000059.3)

• Thank you, this has been modified

4. In the result section represent the mean age along with SD.

• Thank you, this has been added

5. Rephrase the lines 36-49 as: "Two disease causing variants (c.224_227delAAAG and c.5431C>T) in BRCA1 and one (c.5576_5579delTTAA) in BRCA2 was observed in the present study (Table 2). Three variants of undetermined significance (VUS) in BRCA1 (c.536A>G, c.1458T>G, c.1648A>C) were observed in two patients where as one patient had one VUS in BRCA2 (c.1075G>A) (Table 3).

• Thank you this has been modified.
6. In results please specify whether the mutations are observed in familial cases or in sporadic cases.

- Unfortunately due to the retrospective nature of the study, further clinical information is not available for all patients since many were not treated at our institution and were referred specifically for BRCA testing in our referral center

7. In Table 1 add types and stages of cancer

- Unfortunately due to the retrospective nature of the study, further clinical information is not available for all patients since many were not treated at our institution and were referred specifically for BRCA testing in our referral center

8. Delete column Nomenclature protein and Population previously reported form Table 2 and Nomenclature protein and Number of patients with mutation from Table 3. Compile the data of Table 2 and 3 in one table.

- Thank you, due to the different variable we would keep the tables separate for clarity.

9. Append the electropherograms of observed variants.

- Thank you, these have been added

10. Discussion part needs reorganization of data. Correlate the results of present study with published data.

- Thank you, this is the first report of BRCA mutations found in patients from Iraq, we have discussed the literature on the spectrum of BRCA mutations found in the Middle East in general.
11. Keep uniform pattern as per Journal in reference list.

* Thank you, this has been done

Gopeshwar Narayan (Reviewer 2): Comments:

1. The methods are not described in detail. It should be clearly described whether the cDNA was sequenced or genomic DNA was sequenced. I understand that genomic DNA was sequenced. If so, then please provide the list of primer sets for each exon (since PCR products were sequenced).

* Thank you, we have amended the methods section with as much information as we have available.

2. There is no mention of whether the specific mutations were detected in breast cancer patients or ovarian cancer patients, I suggest segregation of mutations in both the cancers.

* Thank you. Unfortunately due to the retrospective nature of the study, further clinical information is not available for all patients since many were not treated at our institution and were referred specifically for BRCA testing in our referral center

3. Representative nucleotide peak pictures showing shifts/gaps/transition for each mutation with sequence should be provided.

* Thank you, these have been added

Dr Deborah Mukherji