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OPEN PEER REVIEW

The Value of Epidermal Growth Factor Receptor Expression in Predicting Aggressive Behaviors of Bladder Neoplasms

Seyed Amir Miratashi Yazdi, Farnoosh Asghari, Hedieh Moradi Tabriz, Behnoud Vesali, Elham Nazar

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 5 Jun 2023 – 6 Jun 2023

Referee 1 Reviewer

MAJOR REVISION 5 Jun 2023

1. The manuscript needs to be edited for grammar and syntax.
2. State the type of the current study in the abstract section.
3. Please check your keywords against the MeSH database. It seems that the keywords need some revisions.
4. What is the difference between your work and that of Afkar A. Badawy (PMID: 28413380), except for the transforming growth factor alpha (TGF-α)? What did you find that differs from her finding?
5. The novelty of your work must be discussed; it seems to be very weak.
6. Describe the groups being compared, including the control groups (positive and negative), in detail.
7. Significant or nonsignificant? Please report the mean (SD) or frequency (%) of each response variable according to each independent variable in the table and text.
8. Please report the exact P-value with three digits in the tables and text.
9. Did you have any missing values in your study? If so, please describe your strategy for solving this problem. It is a critical point for controlling nonresponse bias. Please mention it.
10. First, you must present the demographic variables (such as sex, age, and others) and confounding factors in a table for each group, then compare them, and then present the final result.
11. The immunohistochemical technique should be described in detail.
12. Tumors should be histologically divided into 3 grades (I-III). Please describe this in detail.
13. What classification does tumor staging follow? Please specify this in detail in the text.
14. The meanings of the sentences are not clear (line 16 to 17, line 34 to 36, line 41 to 42, line 111 to 114, and line 123 to 127).
15. You could increase the number of more recent studies in the reference section. You should provide comprehensive and reliable comparisons between your findings and those of previous studies.
16. Additional citations are required to support some of the statements made. Please help improve this article by adding citations to reliable sources.

Associate Editor

MAJOR REVISION 6 Jun 2023

Major Revision

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Hi, Dear Editor

Thanks for your attention, I am pleased to submit the attached revised manuscript for possible publication. Your valuable suggestions were corrected.
Sincerely,

Reviewers' comments:

Reviewer 1:

1. The manuscript needs to be edited for grammar and syntax.

Was improved

2. Write the type of current study in the abstract section.

This cross-sectional study was conducted

3. Please check your Keywords with the Mesh database. It seems that the keywords need some revisions. Check all of the words in the Mesh database and including

EGFR, bladder cancer, prognosis, aggressive behaviors.

4. What is the difference between your work and Afkar A. Badawy (PMID: 28413380) except for the transforming growth factor alpha (TGF-α)? What do you find that is different from her finding?

Also, Badaway et al. showed EGFR expression in 86% of bladder neoplasm with the same methods which was close to our data. They described the considerable connection between EGFR expression and tumor histologic grade and pathologic stage and they could show a relationship between EGFR expression with schistosomal-associated bladder cancer [21]. Our data revealed no significant correlation which may be due to a small population and we couldn’t evaluate schistosomal-associated bladder cancer due to low incidence in our society.

5. The novelty of your work must be discussed, it seems it is so weak.

Was improved.

6. Describe the groups being compared, including the control groups (positive and negative), in detail.

EGFR expression was assessed in bladder neoplasm specimens and compared to normal bladder specimens from random biopsies for other purposes.

Formal, paraffin-embedded fixed sections of normal prostate tissue were used as positive controls for EGFR. Negative controls were performed by replacing the primary antibody with PBS/non-immune mouse serum.

7. Significant or non-significant? Please write (Mean (SD) or frequency (%)) of each response variable according to each independent variable in the table and text.

In male patients, 18 (75%), and in female patients 6 (100%) showed positive staining EGFR. Low-grade tumors showed in 14 (87.5%) patients and high-grade 10 (71.4%) patients had positive EGFR staining. According to Table 3, …….

8. Please mention the exact value of the P-value with three digits in tables and text.

There was no connection between EGFR expression and demographic data and histopathological features including sex (P-value = 0.359), age (P-value = 0.659), surgery type (P-value = 0.39), lymphovascular invasion (P-value = 0.685), tumor size (P-value = 0.294), histologic tumor grade (P-value = 0.171), and pathologic stage (P-value = 0.957).

And Table 3.

9. Did you have any missing value in your study? If it was, please write what was your strategy for solving this problem. It is a critical point for controlling non-response bias. please mention it.

Patients who did not have enough or suitable samples to perform the tests or the patients without available clinical information were excluded from the study. The number of patients required to participate in a study is one of the problems of time-limited studies. Therefore, extending the period may solve the problem.

10. At first you must present the demographic variables (like sex and age and others) and confounding factors in a table according to each group and then compare it then go to the final result.

Tables 1, 2 and 3.

11. The immunohistochemical technique should be described in detail.

The pathology department of Sina Hospital keeps archives of each patient's tumors. Two expert pathologists evaluated the H&E-stained sections of these masses to precisely mark areas of the tumor. IHC was performed in paraffin-fixed, paraffin-embedded 3-µm sections to assess EGFR expression. EGFR expression was investigated using a rabbit anti-EGFR monoclonal antibody (1:50 dilution, duplicate EP22; Master Diag'ostica, Spain). Sections were cut from each block,

deparaffinated with xylene, rehydrated by sequence of decreasing ethanol concentration, and then recovered for antigen in CC1 cytostatic solution (pH 7.4.95◦C; System Y. Ventana) for 60 min and the final time of all recovered solutions cooled for at least 30 min at room temperature (25◦C). Previously, to inhibit endogenous peroxidase, slides were treated for 10 min with 3% H2O2 in methanol. Slides were incubated with monoclonal antibody for 120 min at room temperature and stained with DAKO's Envision detection system. Invention of the color reaction was established with 3,3′-diaminobenzidine tetrahydrochloride (DAB plus; DAKO Glostrup, Denmark) as the substrate, and nuclear contrast was achieved with hematoxylin/water contrasted with ammonia.

12. Tumors should be histologically divided into 3 grades (I-III). Please described in detail.

Tumor grading were including G1, G2, and G3 [10] and according to WHO classification [11] WHO grade 1 lesions that show slight cytologic atypia and mitoses are diagnosed in the WHO/ISUP system as low-grade papillary urothelial carcinomas. WHO grade 2 is a very broad category. It includes lesions that are relatively bland, which in some places are diagnosed as WHO grade 1 to 2; these lesions in the WHO/ISUP system would be called low-grade papillary urothelial carcinoma. In other cases, WHO grade 2 lesions border on higher-grade lesions, which in many institutions are called WHO grade 2 to 3; these lesions in the WHO/ISUP classification system would be called high-grade papillary urothelial carcinoma. Pathological stage is among the most important prognostic factors in bladder cancer.

13. Tumor staging follows what classification. Please specify in detail in the text.

The TNM (tumor, lymph nodes, and metastasis) staging system defines pT1 tumors as those invading the lamina propria but not the muscularis propria; pT2 tumors as those invading the muscularis propria; pT3 tumors as those invading perivesical tissue; and pT4 tumors as those

invading other organ structures (prostate, uterus, vagina, pelvic wall, or abdominal wall) [12].

14. The meaning of the sentences is not clear (line 16 to 17, line 34 to 36, line 41 to 42, line 111 to 114, and line 123 to 127).

Was improved.

15. You could increase the number of more recent studies in the reference section. You should have comprehensive and reliable comparisons between your findings with the other previous studies.

Was corrected.

16. Additional citations are required to support some of the statements made. Please help improve this article by adding citations to reliable sources.

Was improved.

2 Revision 1

2 comments · 26 Jun 2023 – 26 Jun 2023

Referee 1 Reviewer

MINOR REVISION 26 Jun 2023

1- The English in the manuscript has not been revised. In addition, you should highlight all the changes.
2- The manuscript needs correction for spelling and grammar (for instance, line numbers 41 to 42, 67 to 68, 162 to 163, and 174 to 179).
3- Nicholson et al. study described!
For example, research conducted by Nicholson et al. ...
Kim et al. study showed!
A study by Kim et al. revealed ...
Mason et al. study revealed!

Associate Editor

MINOR REVISION 26 Jun 2023

Minor Revision

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Hi, Dear Editor

Thanks for your attention, I am pleased to submit the attached revised manuscript for possible publication. Your valuable suggestions were corrected.
Sincerely,

Reviewers' comments:

Reviewer 1:

1- The English quality of the manuscript has not been revised. In addition, you should highlight all the changes.

Was improved and highlighted.

2- The manuscript needs correction for spelling and grammar. (For instance, line numbers 41 to 42, 67 to 68, 162 to 163, and 174 to 179).

Was corrected and highlighted.

3- Nicholson et al. study described!

For example, research conducted by Nicholson et al. ...

Kim et al. study showed!

A study by Kim et al. revealed ...

Mason et al. study revealed!

A study by Mason, et al. revealed

Research conducted by Chaux et al. found

A study by Wang et al. showed

Research conducted by Kim et al. showed

A study by Badaway et al. showed

3 Revision 2

1 comment · 15 Jul 2023

Associate Editor

ACCEPT 15 Jul 2023

Accept