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Jundishapur Journal of Microbiology
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Peer Review Report for "Aspartyl Proteinase and Phospholipase Activities of *Candida albicans* Isolated From Oropharyngeal Candidiasis in Head and Neck Cancer Patients"

Author(s): Mohsen Ilkhanizadeh-Qomi, Sepideh Nejatbakhsh, Zahra Jahanshiri, Mehdi Razzaghi-Abyaneh

Review Timeline:

Submit Date: 18 May 2020
Revised Date: 22 Sep 2020
Accepted Date: 6 Nov 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Hossein Zarrinfar: Reviewer | Revision (0) 31 Aug 2020

Dear AE,
In the manuscript entitled "Aspartyl proteinase and Phospholipase Activity of Candida albicans Isolated from Oropharyngeal candidosis in Head and Neck Cancer Patients“(ID: 105200).
The authors have described the aspartyl proteinase and phospholipase activity of Candida albicans. This manuscript is interesting and educational. By the way, because of the low number of samples, it can be accepted as a Letter to the editor after revision.
Moreover, there are some grammatical and spelling errors in English too that should be corrected by a person expert. Thus, it may need a minor revision.
The names of enzymes, many variables mentioned in the whole text: Italic and non-italic letters, small and capital, …!!!! It should be revised according to valid journals.
Abstract:
1. “Candida albicans is one of the most important constituents of human normal flora and the cause of opportunistic fungal infection.”: this sentence is unclear and needs revision.
2. “This study was carried out to determine proteolytic and phospholipase activities, with an

emphasis on the gene expressions of C. albicans isolates, obtained from oropharyngeal candidosis in head and neck cancer patients.": this sentence is unclear and needs revision.
3. "32 C. albicans" to be thirty-two C. albicans ..
4. "SAPs genes expressed in all the strains; although, PLBs genes were not expressed in four isolates. ": this sentence is unclear and needs a revision.
5. "Our findings demonstrate that C. albicans strains isolated from oropharyngeal candidosis patients in Iran had strong proteolytic and phospholipase activities, and were able to express high mRNA levels of the encoding genes of these enzymes.": this sentence is too long, unclear, and needs revision.
Background:
1. "opportunistic fungal infections (1) Candida albicans is also one", to be revised and add the new related references: opportunistic fungal infections (1). C. albicans is also one of the most important known human pathogens that can cause various clinical forms (a, b and c references):
a. Recurrent Vulvovaginal Candidiasis: The Causative agents, Clinical signs and susceptibility to fluconazole in Gonabad City, The Northeast of Iran. Current Women`s Health Reviews. 2020; 16(1):46-51.
b. Rapid detection of Candida species in bronchoalveolar lavage fluid from patients with pulmonary symptoms. Braz J Microbiol. 2016; 47(1): 172-6.
c. Molecular Identification of Candida species isolated from patients with vulvovaginitis for the first time in Mashhad. Iranian Journal of Obstetrics, Gynecology and Infertility. 2017; 20(4): 50-57.
2. "extracellular hydrolytic enzymes in candida species (2).", to be extracellular hydrolytic enzymes in Candida species (2).
3. "It has been demonstrated that the products of Sap9-10 genes are considered to attach to the cell membrane while SAP 1-8 are released into the extracellular spaces (5).", this sentence is unclear and needs revision.
4. "Altogether, SAPs enzymes represented important roles in colonization and incursion and causing of different kind of Candida infections. (7).", this sentence is unclear and needs revision.
5. "The most activity of phospholipase B in C. albicans belongs to PLB1, whereas PLB2 contributes slightly. It has been demonstrated that PLB1-deficient strains are only able to produce a little phospholipase B activity (10).", this sentence is unclear and needs revision.
6. "Oropharyngeal candidosis (OPC)", to be: Oropharyngeal candidiasis (OPC)
7. "It is envisaged that our data can improve our knowledge regarding the virulence factors of C. albicans isolates that cause OPC in HNC patients and can be used toward more effective treatments strategies for these patients.", this sentence is unclear and needs a revision. How can this information be used to treat patients effectively?
8. "Here, we evaluated the expressions of SAP1, SAP3, PLB1 and PLB2 genes in C. albicans strains obtained from OPC in HNC patients.", this sentence is repetitive!!
Methods:
9. "32 clinical C. albicans isolates,", to be: thirty-two clinical C. albicans isolates,
10. There are not any documents for results of MLST and ITS sequencing (primers information, ..)!!! Please add them.
11. "Ruchell et al. (14).", et al. to be italic inside all text.
12. "After incubation, the clearance halo …. high Pz value represents low enzymatic

activity.", this sentence is unclear and needs revision. Also, 1, 0.64 what mean? Unit of measurement?

13. "The culture medium was SDA that was composed of specific compounds.", what do you mean? What is its role?

14. "Total RNA was extracted using Guanidinium isothiocyanate (GITC) reagent from fresh yeast cells. Total RNA was treated by RNase-free DNase (Thermo Fisher Scientific, USA) (16).", this procedure is unclear!

15. "Total RNA was treated", how did you measure?

16. "The specific primers set are shown in Table 1.", Are these primers designed by the authors themselves?

Results:

17. "28 strains (87.5 %)", to be twenty-eight strains (87.5 %).

18. "SAPs and PLBs genes expression by Real-Time PCR", in this section, the authors have stated some information about SAP 2! But, the aim of the study is not about it!!

19. "genes in all Candida isolates", to be genes in all Candida isolates.

20. "C. albicans SC5314", reasons of use this strain? Role?

21. "Real-Time PCR amplification identified 4 patterns for SAP1-3 and also 4 patterns for PLB1-2 expressions in the C. albicans isolates.", this sentence is unclear and needs revision. What do you mean?

22. "SAP1 in the two major clades, except in one strain, was expressed in the all so that the highest expression was in the first major clade.", this sentence is unclear and needs a revision.

Discussion:

23. "Moreover, an important relationship between pathogenicity and proteinase activity was found in Candida spp. and duration of C. albicans infection is related to the total number of virulence factors (19, 20).", this sentence is unclear and needs a revision.

24. "well as SAP1, SAP3, LPB1 and LPB2 gene", LPB1 and LPB2 what mean!!

25. "In the present study, we showed that after SAP2, SAP1 had the highest level of expression among the SAPs genes as SAP3 was expressed in 62.5 % (n = 20) and SAP1 was expressed in 87.5 % (n = 28) of the oral clinical isolates of C. albicans.", this sentence is unclear and needs a revision. Moreover, your discussion should be on SAP 1 and 3.

26. "We found 4 patterns based on SAP1-3 expressions (Table 2) while the pattern in which all the genes were being expressed was the most abundant one (53.12%, n = 17).", this sentence is unclear and needs a revision.

27. "Santosh et al. have observed in 2014 that the rate of production of secreted aspartyl proteinase enzyme (strain non-albicans) has a significant difference (0.17-0.61 µg / ml) with C. albicans strains (0.56 to 0.82 µg / ml) which shows that C. albicans strain is more potent secretors of aspartyl proteinase enzymes than the non-albicans strains (23).", this sentence is too long and unclear and needs a revision.

28. "though the expression of SAP3 gene is high in active mucosal infections in compare with asymptomatic colonization, it cannot be considered as a more often detectable element of the SAP gene family.", this sentence is unclear and needs a revision.

29. "in the carriers (6). member, what mean!!

30. "Since C. albicans is the most common species which is able to colonize and infect oral tissue effectively in these patients, extensive comparative studies from large groups with the various sources of infection are necessary for determination of the relationship between

virulence factors and MLST pattern and inclusive profile of virulence in C. albicans isolates.", this sentence to be omitted and it should be according to aims and results.
31. Figure 1 and 2, results of SAP 2 to be omitted. Because it is not related to study aims. Unit of measurement of the vertical axis? Horizontal axis, "species" to be Candida albicans strains.
32. Figure 3, results of SAP 2 to be omitted. Because it is not related to study aims.
33. Table 1, the title is uncompleted and unclear.
34. Tables 2 and 3 should be merged. Explain the patterns.
Thanks.

REFEREE: Reviewer | **Revision** (0) 8 Sep 2020

Dear AE,
The manuscripts describe the expression of Aspartyl proteinase and phospholipase genes in Candida albicans isolated from oropharyngeal candidosis in Head and Neck Cancer Patients.
Major modifications:
1- I believe that gene expression is a process of transcription, not the translation, and not post-translation modifications, so in this study, the expression of each gene had to be calculated between candida isolates from head and neck cancer (HNC) patients and isolates from other sites of patients or who were asymptomatic carriers (volunteers or individuals). Why the authors just calculate the expressions of SAP1, SAP3, PLB1, and PLB2 genes of C. albicans isolates, obtained from OPC in HNC patients??
2- Please check all references again, add and replace the correct references related to written content.
Minor modifications:
In addition, the authors should consider the suggestions listed below:
Abstract
• ''Several factors have been identified which contribute to the pathogenic potential of this fungus''. Are you sure that hydrolytic enzymes are the main virulence factor in the pathogenesis of Candida species? Please rephrase and clarify it in abstract and background parts.
• ''Authors mention; our findings demonstrate that C. albicans strains isolated from oropharyngeal candidosis patients in Iran had strong proteolytic and phospholipase activities''. Did the authors recover these 32 isolates from different parts of Iran? If yes, please point to those in detail. If No, the authors cannot generalize these results to Iran.
Background
• Please add a dot after reference 1
• ''the greatest identified secretory enzymes produced by Candida spp.'' Replace producted with produced.
• ''It has been demonstrated that the products of Sap9-10 genes''. Replace Sap9-10 with SAP9-10.
• ''The infection may cause critical''. Replace cause with cause.
• ''It has been demonstrated that C. albicans is the most common…..''. Repetitive sentences

Methods

• According to sampling from patients, please add a code of ethics

• Please add all company and country names of supplied instruments and materials.

• ‘’typed by MLST were used in this study.’’ please put the related reference.

• ‘’The final identification was done by ITS sequencing method’’. Please add the accession numbers of sequences.

• ‘’cultured as spot and incubated at 37 ◦C for 5 days’’. Replace 37 ◦C with 37 °C.

• ‘’was determined using the mean of precipitation zone (Pz) values’’. Please add related references and required abbreviations. Ex: Proteinase activity (Prz value) or phospholipase activity (Pz value).

• ‘’The culture medium was SDA that was composed’’. For the first time, SDA should not use with abbreviations. Modify to complete name.

• ‘’The average of Pz was measured for all the’’. Replace measured with measured.

• ‘’on Sabouraud Dextrose Agar (Merck, Germany) at 28 °C for 48 h. Total RNA was extracted using….’’. Replace 48 h. Total with 48 h, total.

• ‘’by RNase-free DNase (Thermo Fisher Scientific, USA) (16)’. Please recheck the references and put the exact reference.

• ‘’each reaction was prepared in a final volume of 25 µl’. Replace µl with µL in all parts.

• ‘’The specific primers set are shown in Table 1’’. Please put all references to the primer

• ‘’PCR were as follow 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min’ (17)’’. Please recheck the reference and put the exact reference.

• Please clarify, based on which formula the obtained data were analyzed?

Results

• ‘’All of the Candida strains represented proteinase activity’’. Replace strains with strains.

• ‘’According to Figure 3, in the three major clades of MLST, genes expression was different’’. Please put the exact reference related to MLST.

• ‘’showed a moderate and 4 isolates (12.5 %) had no phospholipase activity (Table 2, Table 3)’’. It is suggested that Tables 2 and 3 be merged.

• ‘’Figure .1: Analysis of SAP2 was not the aim of the study!!. Please add proper figures related to SAP1 and SAP3

• ‘’Figure.1 The expression of sap1-sap3 genes in 32 C. albicans strains’’. Replace sap1-sap3 with SAP1 and SAP3.

• ‘’Figure.2 The expression of plb1-plb2 genes in 32 C. albicans strains’’. Replace plb1-plb2 with PLB1 and PLB2.

Discussion

• ‘’that SAP1 and SAP3 genes are preferentially expressed and have principle roles in oral candidosis….’’. This content can be true if the authors had a control group. Please be more precise.

• ‘’In the present study, we showed that after SAP2, SAP1 had the highest level of expression among the SAPs genes as SAP3 was expressed’’. Analysis of SAP2 was not done in this work, so it should be with reference and comparison mode.

• ‘’SAP3 gene is high in active mucosal infections in compare’’. Replace Comair with compare.

• ‘’with asymptomatic colonizations’’,. Delete space between with and asymptomatic.

• ‘’SAP3 expression is more common in patients with vaginal infection or in the carriers (6). Member’’. Delete Member

• ‘’was more than PLB2 in C. albicans isolates and similar to previous studies’’. The authors point to previous studies but put one reference. Please add the other related references.
• ‘’ and comparison between three major clades derived from MLST typing method’’. Please put the related reference.
Thanks.

REFEREE: Associate Editor | **Revision (0)** 9 Sep 2020

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

REFEREE: EIC | **Revision (0)** 9 Sep 2020

Dear Authors,
Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments. Please answer point by point to the reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers’ queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding.
When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made a point by point, and provide suitable rebuttals for any comments not addressed. In addition, JJM INSTRUCTION attached to the mail, Pleases find it.
Note:
Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. http://single.demo.pub/en/knowledgebase/category/tree.html#orcid.html
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Ref. No.: Jundishapur J Microbiol | ID: 105200]

To: Prof. Ali Zarei Mahmoudabadi

Dear Prof. **Zarei Mahmoudabadi** September 22, 2020
Editor-in-Chief
Jundishapur journal of microbiology

Authors are thankful to reviewers for their critical view and suggestions for improvement of the manuscript. The manuscript is re-structured keeping all the critical comments of the reviewers. All the suggestions of the reviewers have been incorporated in the revised manuscript (text with **Yellow highlight**) as follows:

Reviewer 1:

In the manuscript entitled "Aspartyl proteinase and Phospholipase Activity of Candida albicans Isolated from Oropharyngeal candidosis in Head and Neck Cancer Patients"(ID: 105200).
The authors have described the aspartyl proteinase and phospholipase activity of Candida albicans. This manuscript is interesting and educational. By the way, because of the low number of samples, it can be accepted as a Letter to editor after revision.
Moreover, there are some grammatical and spelling errors in English too that should be corrected by a person expert. Thus, it may need a minor revision.

-language editing was done in the whole manuscript.

The names of enzymes, many variables mentioned in the whole text: Italic and non-italic letters, small and capital, …!!!! It should be revised according to valid journals.

-the name of enzymes was corrected according to valid journal in the whole manuscript.

Abstract:
1. "Candida albicans is one of the most important constituents of human normal flora and the cause of opportunistic fungal infection.": this sentence is unclear and needs a revision.

-the sentence was corrected. The abstract section, lines 1 and 2.

2. "This study was carried out to determine proteolytic and phospholipase activities, with an emphasis on the gene expressions of C. albicans isolates, obtained from oropharyngeal candidosis in head and neck cancer patients.": this sentence is unclear and needs a revision.

-the sentence was corrected. The abstract section, lines 4 to 6.

3. “32 C. albicans” to be thirty two C. albicans ..

-the sentence was corrected. The abstract section, line 7.

4. “SAPs genes expressed in all the strains; although, PLBs genes were not expressed in four isolates. ”: this sentence is unclear and needs a revision.

-the sentence was corrected. The abstract section, lines 12 and 13.

5. “Our findings demonstrate that C. albicans strains isolated from oropharyngeal candidosis patients in Iran had strong proteolytic and phospholipase activities, and were able to express high mRNA levels of the encoding genes of these enzymes.”: this sentence is too long, unclear and needs a revision.

-the sentence was corrected. The abstract section, lines 14 and 15.

Background:

1. “opportunistic fungal infections (1) Candida albicans is also one”, to be revised and add the new related references: opportunistic fungal infections (1). C. albicans is also one of the most important known human pathogens that can cause various clinical forms (a, b and c references):

a. Recurrent Vulvovaginal Candidiasis: The Causative agents, Clinical signs and susceptibility to fluconazole in Gonabad City, The Northeast of Iran. Current Women`s Health Reviews. 2020; 16(1):46-51.

b. Rapid detection of Candida species in bronchoalveolar lavage fluid from patients with pulmonary symptoms. Braz J Microbiol. 2016; 47(1): 172-6.

c. Molecular Identification of Candida species isolated from patients with vulvovaginitis for the first time in Mashhad. Iranian Journal of Obstetrics, Gynecology and Infertility. 2017; 20(4): 50-57.

-the sentence was corrected and the references were added. The background section, page 1, line 3; reference section, No. 2, 3 and 4.

2. “extracellular hydrolytic enzymes in candida species (2).”, to be: extracellular hydrolytic enzymes in Candida species (2).

-the sentence was corrected. . The background section, page 1, lines 5.

3. "It has been demonstrated that the products of Sap9-10 genes are considered to attach to the cell membrane while, SAP 1-8 are released into the extracellular spaces (5).", this sentence is unclear and needs a revision.

-the correction was done. The background section, page 1, lines 7, 8 and 9.

4. "Altogether, SAPs enzymes represented important roles in colonization and incursion and causing of different kind of Candida infections. (7).", this sentence is unclear and needs a revision.

-the correction was done. The background section, page 1, lines 14 and 15.

5. "The most activity of phospholipase B in C. albicans is belongs to PLB1, whereas PLB2 contributes slightly. It has been demonstrated that PLB1-deficient strains are only able to produce a little phospholipase B activity (10).", this sentence is unclear and needs a revision.

-the correction was done. The background section, page 1, lines 17, 18 and 19.

6. "Oropharyngeal candidosis (OPC)", to be: Oropharyngeal candidiasis (OPC)

-the correction was done. The background section, page 1, second paragraph, lines 20.

7. "It is envisaged that our data can improve our knowledge regarding the virulence factors of C. albicans isolates that cause OPC in HNC patients and can be used toward more effective treatments strategies for these patients.", this sentence is unclear and needs a revision. How can this information be used to treat patients effectively?

-the sentence was corrected. The background section, page 3, lines 2-8.

8. "Here, we evaluated the expressions of SAP1, SAP3, PLB1 and PLB2 genes in C. albicans strains obtained from OPC in HNC patients.", this sentence is repetitive!!
-the correction was done. Page 3, objective section.

Methods:

9. "32 clinical C. albicans isolates,", to be: thirty two clinical C. albicans isolates,

-the sentence was corrected. Page 3, the methods section, clinical isolates section, line 2.

10. There are not any documents for results of MLST and ITS sequencing (primers information, ..)!!! Please add them.

-MLST data was mentioned in our previous study, the reference was added in the manuscript. Page 2, Methods section, clinical isolates section, line 4, reference No. 16.

-the information of ITS sequencing was added. Page 3, the methods section, clinical isolates section, page 3, line 8, 9 and 10, and page 4, line 1and 2.

11. "Ruchell et al. (14).", et al. to be italic inside the all text.

-the correction was done. Page 4, proteolityc activity section, line 2.

12. "After incubation, the clearance halo …. high Pz value represents low enzymatic activity.", this sentence is unclear and needs a revision. Also, 1, 0.64 what means? Unit of measurement?

-the sentences were corrected. Page 4, proteolityc activity section, lines 4, 5, 6, 7,8 and 9.

13. "The culture medium was SDA that was composed of specific compounds.", what do you mean? What is its role?

-the sentence was corrected. Page 5, lines 2, 3 and 4.

14. "Total RNA was extracted using Guanidinium isothiocyanate (GITC) reagent from fresh yeast cells. Total RNA was treated by RNase-free DNase (Thermo Fisher Scientific, USA) (16).", this procedure is unclear!

-the sentences were corrected. Page 5, RNA extraction section, lines 1, 2, 3 and 4.

15. "Total RNA was treated", how did you measure?

-the description was added. Page 5, RNA extraction section, lines 5 and 6.

16. "The specific primers set are shown in Table 1.", Are these primers designed by the authors themselves?

-the reference was added. Reference No. 9.

Results:

17. "28 strains (87.5 %)", to be twenty eight strains (87.5 %) ..

-the sentence was corrected in the text. Page 6, result section, line 1.

18. "SAPs and PLBs genes expression by Real-Time PCR", in this section, the authors have stated some information about SAP 2! But, the aim study is not about it!!

-the section was corrected. The data of *SAP2* was deleted. Page 6, results section, *SAPs* and *PLBs* genes expression … section, lines 10, page 7, the first line. Figure 1, Figure 3, Table 2.

19. "genes in all Candida isolates", to be genes in all Candida isolates ..

-the correction was done. Page 6, results section, *SAPs* and *PLBs* genes expression … section, line 1.

20. "C. albicans SC5314", reasons of use this strain? Role?

-the description was added. Page 6, results section, *SAPs* and *PLBs* genes expression … section, lines 2, 3, 4 and 5.

21. "Real-Time PCR amplifications identified 4 patterns for SAP1-3 and also 4 patterns for PLB1-2 expressions in the C. albicans isolates.", this sentence is unclear and needs a revision. What do you mean?

-the sentence was corrected. Page 6, results section, *SAPs* and *PLBs* genes expression … section, lines 8 and 9.

22. "SAP1 in the two major clades, except in one strain, was expressed in the all, so that the highest expression was in the first major clade.", this sentence is unclear and needs a revision.

-the correction was done. Page 7, the first paragraph, lines 5, 6 and 7.

Discussion:

23. "Moreover, an important relationship between pathogenicity and proteinase activity was found in Candida spp. and duration of C. albicans infection is related to the total number of virulence factors (19, 20).", this sentence is unclear and needs a revision.

-the revision was done. Page 7, discussion section, first paragraph, lines 2, 3,4 and 5.

24. "well as SAP1, SAP3, LPB1 and LPB2 gene", LPB1 and LPB2 what mean!!

-the sentence was corrected. Page7, discussion section, first paragraph, lines 7 and 8.

25. "In the present study, we showed that after SAP2, SAP1 had the highest level of expression among the SAPs genes as SAP3 was expressed in 62.5 % (n = 20) and SAP1

was expressed in 87.5 % (n = 28) of the oral clinical isolates of C. albicans.", this sentence is unclear and needs a revision. Moreover, your discussion should be on SAP 1 and 3.

-the correction was done in the text. Discussion section, page 7, the last line, page 8, lines 1and 2.

26. "We found 4 patterns based on SAP1-3 expressions (Table 2) while the pattern in which all the genes were being expressed was the most abundant one (53.12%, n = 17).", this sentence is unclear and needs a revision.

-Table 2 was revised and the sentence was corrected. Discussion section, page 8, lines 2, 3, 4and 5.

27. "Santosh et al. have observed in 2014 that the rate of production of secreted aspartyl proteinase enzyme (strain non-albicans) has a significant difference (0.17-0.61 µg / ml) with C. albicans strains (0.56 to 0.82 µg / ml) which shows that C. albicans strain are more potent secretors of aspartyl proteinase enzymes than the non-albicans strains (23).", this sentence is too long and unclear and needs a revision.

-the correction was done in the text. Page 8, second paragraph, lines 9, 10, 11 and 12.

28. "though the expression of SAP3 gene is high in active mucosal infections in compair with asymptomatic colonizations, it cannot be considered as more often detectable element of the SAP gene family.", this sentence is unclear and needs a revision.

-the sentence was corrected. Page 8, the last paragraph, lines 3, 4 and 5.

29. "in the carriers (6). member, what means!!

-the revision was done.

30. "Since C. albicans is the most common species which is able to colonize and infect oral tissue effectively in these patients, extensive comparative studies from large groups with various source of infection are necessary for determination of relation between virulence factors and MLST pattern and inclusive profile of virulence in C. albicans isolates.", this sentence to be omitted and it should be according to aims and results.

-the sentence was deleted and the paragraph was rewritten. Conclusions section, lines 3, 4, 5, 6, 7, 8 and 9.

31. Figure 1 and 2, results of SAP 2 to be omitted. Because, it is not related to study aims. Unit of measurement of vertical axis? Horizontal axis, "species" to be Candida albicans strains.

-the data of *SAP2* was omitted in Figure 1 and Figure 3. Unit of measurement was corrected in Figure 1 and Figure 2.

32. Figure 3, results of SAP 2 to be omitted. Because, it is not related to study aims.

-Figure 3 was corrected.

33. Table 1, the title is uncompleted and unclear.

-the title of Table 1 was revised.

34. Tables 2 and 3 should be merged. Explain about the patterns.

-Table 2 and 3 were merged and the correction was done in the Table (Table 2).

Reviewer 2:

Dear authors

The manuscripts describe the expression of Aspartyl proteinase and phospholipase genes in Candida albicans isolated from oropharyngeal candidosis in Head and Neck Cancer Patients.

Major modifications: 1- I believe that gene expression is a process of transcription, not translation, and not post translation modifications, so in this study the expression of each gene had to be calculated between candida isolates from head and neck cancer (HNC) patients and isolates from other sites of patients or who were asymptomatic carriers (volunteers or individuals). Why the authors just calculated the expressions of SAP1, SAP3, PLB1 and PLB2 genes of C. albicans isolates, obtained from OPC in HNC patients??

We agree with the editor for the importance of data analysis for *C. albicans* strains from control group. Actually, we did this important issue for *SAPs* and *PLBs* genes expression to provide comparative results for making a reliable conclusion. Five isolates from non-OPC patients were considered as the control of gene expression. The results of *SAPs* and *PLBs* genes expression in non-OPC patients were added in the manuscript.

Page 3, clinical isolates section, line 4 and 5, page 8, the first paragraph, lines 2 and 3. Page 9, lines 11 and 12. . Figure 1 and Figure 2, page 14 and 15, figure legend 1 and 2.

2- Please check all references again, add and replace the correct references related to written content.

All the references were checked in whole manuscript.

Minor modifications:

In addition, the authors should consider the suggestions listed below:
Abstract
• ''Several factors have been identified which contribute to the pathogenic potential of this fungus''. Are you sure that hydrolytic enzymes are the main virulence factor in pathogenesis of Candida species? Please rephrase and clarify it in abstract and background parts.

-the sentence was corrected. Abstract section, lines 2 and 3.

• ''Authors mention; our findings demonstrate that C. albicans strains isolated from oropharyngeal candidosis patients in Iran had strong proteolytic and phospholipase activities''. Did authors recover these 32 isolates from different parts of Iran? If yes, please point to those in details. If No, the authors cannot generalize these results to Iran.

- **The description and the reference were added in the text. Page 3, method section, lines 2 and 3. (The details of geographic distribution of the isolates were mentioned in our previous study (reference No. 16).**

Background
• Please add dot after reference 1

-the correction was done. Page 2, background section, line 2.

• ''the greatest identified secretory enzymes producted by Candida spp.'' Replace producted with produced.

-the language editing was done in whole manuscript and the sentence was changed.

• ''It has been demonstrated that the products of Sap9-10 genes''. Replace Sap9-10 with SAP9-10.

-the correction was done. Page 2, background section, lines7, 8 and 9.

• ''The infection may cuase critical''. Replace cuase with cause.

-the correction was done. Page 2, second paragraph, line 2.

• ''It has been demonstrated that C. albicans is the most common…..''. Repetitive sentences

-the repetitive sentence was deleted.

Methods

• According to sampling from patients, please add code of ethics

-the code of ethic was added. Page 3, method section, lines 1 and 2.

• Please add all company and country name of supplied instruments and materials.

-Chemical and instruments section was added. Page 4, chemicals and instruments section.

• ''typed by MLST were used in this study.'' please put the related reference.
-the reference was added. Page 3, method section, reference No.16.

• ''The final identification was done by ITS sequencing method''. Please add the accession numbers of sequences.
-the accession numbers were added. Page 3, the last line, page 4, lines 1 and 2.

• ''cultured as spot and incubated at 37◦C for 5 days''. Replace 37◦C with 37 °C.
-the correction was done. Page 4, proteolytic activity section, line 3.

• ''was determined using the mean of precipitation zone (Pz) values''. Please add related reference and required abbreviation. Ex: Proteinase activity (Prz value) or phospholipase activity (Pz value).
-the correction was done. Page 4, proteolytic activity section, lines 4, 5, 6, 7,8 and 9.

• ''The culture medium was SDA that was composed''. For the first time SDA should not use with abbreviation. Modify to complete name.

-the correction was done. Page 4, chemical and instruments section, line 1. Page 5, line 2.

• ''The average of Pz was measured for all the''. Replace measaured with measured.
-the correction was done. Page 5, first paragraph, line 7.

• ''on Sabouraud Dextrose Agar (Merck, Germany) at 28 °C for 48 h. Total RNA was extracted using….''. Replace 48 h. Total with 48 h, total.
-the paragraph was revised. Page 5, RNA extraction section.

• ''by RNase-free DNase (Thermo Fisher Scientific, USA) (16)'. Please recheck the references and put the exact reference.

-the exact reference was added. Page 5, RNA extraction section, reference No. 20, reference section, page 11, reference No. 20.

• *''each reaction was prepared in final volume 25 µl'. Replace µl with µL in all parts.*
-the correction was done. Page 5, the last paragraph, line 3.

• *''The specific primers set are shown in Table 1''. Please put all references of the primer*
-the reference was added. Page 5, the last paragraph, line 3.

• *''PCR were as follow: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min' (17)''. Please recheck the reference and put the exact reference.*
-the exact reference was added. Page 5, the last paragraph, line 4, (reference number 9).

• *Please clarify, based on which formula the obtained data were analyzed?*
-the correction was done. Page 6, line 1, 2 and 3.

Results
• *''All of the Candida straines represented proteinase activity''. Replace straines with strains.*
-the correction was done. Page 6, result section, line 1.

• *''According to Figure 3, in the three major clades of MLST, genes expression was different''. Please put the exact reference related to MLST.*
-the correction was done. Page 7, the first paragraph, line 4, reference No. 16.

• *''showed a moderate and 4 isolates (12.5 %) had no phospholipase activity (Table 2, Table 3)''. It is suggested that Tables 2 and 3 be merged.*
-the correction was done. Table 2.

• *''Figure .1: Analysis of SAP2 was not the aim of the study!!. Please add proper figure related to SAP1 and SAP3*
-the correction was done. Figure 1.

• *''Figure .1 The expression of sap1-sap3 genes in 32 C. albicans strains''. Replace sap1-sap3 with SAP1 and SAP3.*
-the correction was done. Figure 1.

• *''Figure .2 The expression of plb1-plb2 genes in 32 C. albicans strains''. Replace plb1-plb2 with PLB1 and PLB2.*
-the correction was done. Figure 2.

Discussion
• *''that SAP1 and SAP3 genes are preferentially expressed and have principle roles in*

oral candidosis….". This content can be true if the authors had a control group. Please be more precise.
-the correction was done and control group were added. Page 3, method section, line 4 and 5.

• *''In the present study, we showed that after SAP2, SAP1 had the highest level of expression among the SAPs genes as SAP3 was expressed". Analysis of SAP2 was not done in this work, so it should be with reference and comparison mode.*
-According to the first reviewer comment, the information about *SAP2* was deleted.

• *''SAP3 gene is high in active mucosal infections in compare". Replace compair' with compare.*
-the sentence was revised. Page 8, the last paragraph, lines 9, 10 and 11.

• *''with asymptomatic colonizations",. Delete space between with and asymptomatic.*
-the sentence was revised. Page 8, the last paragraph, lines 9, 10 and 11.

• *''SAP3 expression is more common in patients with vaginal infection or in the carriers (6).*
Member". Delete Member
-the deletion was done.

• *''was more than PLB2 in C. albicans isolates and similar to previous studies". The authors point to previous studies, but put one reference. Please add the other related references.*
-the reference was added. Page 9, line 9, reference No. 34. Reference section, reference No. 34.

• *'' and comparison between three major clades derived from MLST typing method". Please put the related reference.*
-the reference was added. Page 9, line 15, the reference No. 16.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 6 Nov 2020

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