PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	The role of Hypoxic-inducible Factor (HIF1α) and Aldolase C Protein in Endometrial Carcinogenesis: A retrospective Study of 279 Patients.
AUTHORS	Mhawech-Fauceglia, Paulette ; wang, dan; samrao, damanzoopinder; Menesses, Teodulo; Godoy, heidi; ough, faith; Lele, shahikant; liu, song; pejovic, tanja

VERSION 1 - REVIEW

REVIEWER	Dr Logan Walker Research Fellow University of Otago New Zealand I declare no competing interests
REVIEW RETURNED	19-Jun-2012

GENERAL COMMENTS	Minor comments: - Page 7, line 1 - "HF1" should be "HIF1" - Page 7, line 16 - "2-" should be (2) - Table 3 - "IHFa" should be "HIF1a" - Table 3 - It is unclear why FIGO_grade 2 and Nuclear_grade 2 percentages do not fall between grade 1 and 3. Would this be expected, and if so, how do the authors explain this anomaly? - Page 15, line 8 - "anaerobic" sould be "aerobic"?

REVIEWER	Prim. Priv. Doz. DDr. Hermann Brustmann Director, Dept. of Pathology Landesklinikum Baden-Mödling Sr.Maria Restitutagasse 12, A-2340 Mödling Austria I have no competing interests to declare. Hermann Brustmann, M.D., Ph.D.
REVIEW RETURNED	08-Jul-2012

GENERAL COMMENTS	The authors investigated HIF1α and aldolase C protein expression in endometrial cancer. The study is generally well written and carefully designed. Limitations of the study are addressed appropriately; however, in a patient cohort defined by a certain time interval it has to be expected that the number of type II cancers is rather small and that high tumor stages do not prevail. There are some minor points I would like to raise.

	- Page 8, histological evaluation: The authors apparently graded the tumors twice. They used the FIGO grading system and, in another step, evaluated nuclear grade. There are some suggestions that these two approaches to grade may be combined and that high nuclear grade may increase the grade by 1 point. However, the method to reach a nuclear grade should be explained in detail for each of the three different grades or quoted from previous literature. Why were the FIGO 1989 guidelines to stage employed instead of the recent FIGO staging? - Page 8, immunohistochemistry: The authors merely depended on staining intensity to reach their immunohistochemical scores. Were there any positive controls to refer to strong staining intensity? - Tables: The median values are surely interesting. Information may be enhanced by mean values. - Page 11: the authors claim that “stain intensity was diffuse and homogenous throughout the tumor”. Is this also true for superficial parts and the deep aspects at the invasive front of one and the same tumor? Were there no differences for different patterns of invasion (e.g., MELF)? - Figures: The figures show appropriate lesions. However, they indicate the above discussed weakness of the staining evaluation system. In Figure 3 there is no diffuse nuclear staining for HIF1α, many nuclei remain unstained in both specimens. This seems also true for Fig.2B with cytoplasmic aldolase staining; however, this micrograph is somewhat dark. Since the reader does not know the qualities of weak staining it remains unclear whether cytoplasmic aldolase staining is weak or non-specific in Fig 1A. The authors may choose for a percentage of cells as a cut-off level for positive and diffuse staining.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Dr Logan Walker
Research Fellow
University of Otago
New Zealand

I declare no competing interests

Minor comments:
- Page 7, line 1 - "HF1" should be "HIF1" Done
- Page 7, line 16 - "2-" should be (2) Done
- Table 3 - "IHFa" should be "HIF1a" Done
- Table 3 - It is unclear why FIGO_grade 2 and Nuclear_grade 2 percentages do not fall between grade 1 and 3. Would this be expected, and if so, how do the authors explain this anomaly? We graded the endometrial cancer based on the architectural FIGO staging system criteria and we graded based on the nuclear criteria. We also added the nuclear criteria in the materials and methods section

- Page 15, line 8 - "anaerobic" sould be "aerobic"? Yes, we did the correction.

Reviewer: Prim. Priv. Doz. DDr. Hermann Brustmann
Director, Dept. of Pathology
Landesklinikum Baden-Mödling
Austria

Q1:- Page 8, histological evaluation: The authors apparently graded the tumors twice. They used the FIGO grading system and, in another step, evaluated nuclear grade. There are some suggestions that these two approaches to grade may be combined and that high nuclear grade may increase the grade by 1 point. However, the method to reach a nuclear grade should be explained in detail for each of the three different grades or quoted from previous literature. Why were the FIGO 1989 guidelines to stage employed instead of the recent FIGO staging?
A2: We explained in details the nuclear grading in the section of materials and methods. Please see our answer for the first reviewer; the old FIGO staging was used because most of our cases were from 2009-2000.Thus, we though was more appropriate to use the old FIGO staging system instead of the 2010 FIGO staging.

Q2:- Page 8, immunohistochemistry: The authors merely depended on staining intensity to reach their immunohistochemical scores. Were there any positive controls to refer to strong staining intensity?
A2: We mentioned the positive control in MM.

Q2:- Tables: The median values are surely interesting. Information may be enhanced by mean values.
A2: Our statistician (D.Wang and S.Liu) did calculate the mean values and in their results, the mean values and the median values were very close. So, we will just keep the median as it was already there.

Q3- Page 11: the authors claim that “stain intensity was diffuse and homogenous throughout the tumor”. Is this also true for superficial parts and the deep aspects at the invasive front of one and the same tumor? Were there no differences for different patterns of invasion (e.g., MELF)?
A3: Due to the confusion create dit by this sentence, We canceled it. No, we did not look at the invasive front of the tumor.

Q4- Figures: The figures show appropriate lesions. However, they indicate the above discussed weakness of the staining evaluation system. In Figure 3 there is no diffuse nuclear staining for HIF1α, many nuclei remain unstained in both specimens. This seems also true for Fig.2B with cytoplasmic aldolase staining; however, this micrograph is somewhat dark. Since the reader does not know the qualities of weak staining it remains unclear whether cytoplasmic aldolase staining is weak or non-specific in Fig 1A. The authors may choose for a percentage of cells as a cut-off level for positive and diffuse staining
A4: we changed the images to reflect the staining pattern better.
We hope that our reply will satisfy the reviewers’ comments.

VERSION 2 – REVIEW

REVIEWER	Prim. Priv. Doz. DDr. Hermann Brustmann Dept.of Pathology Landesklinikum Baden-Moedling Sr. Maria Restitutagasse 12 A-2340 Moedling Austria I have no competing interests to declare.

REVIEW RETURNED	14-Jul-2012

- The reviewer completed the checklist but made no further comments.