Increased PTEN gene expression in patients with endometrial carcinoma from areas of high risk depleted uranium exposure

Alaa Salah Jumaah¹, Hawraa Sahib Al-Haddad², Liwaa Hussein Mahdi¹, Emad Hatem³, Asaad Abdul Hamza Al-Janabi¹, Katherine McAllister⁴ and Akeel Abed Yasseen* ¹

Abstract

Objective: Investigate PTEN gene expression and tumor aggressiveness in endometrial carcinoma specimens from patients living in either areas of depleted uranium [DU] pollution or unpolluted regions to determine any evidence for the effect of war pollution on the rising trends of cancer incidence in Iraq.

Results: Tumor PTEN gene expression was significantly increased in patients living in the areas of high risk DU exposure, in comparison to patient tumors from low risk areas [P = 0.001]. The age distribution between the potentially DU exposed (55.09 ± 1.24) and unexposed subjects 56.38 ± 1.18) was not significant [P = 0.45]. Endometrial carcinoma aggressiveness was equivalent in both subject groups, with no significant differences in either tumour grade and [P = 0.286] stage distribution [P = 0.98]. Finally, there were no significant differences between the potentially exposed and unexposed subjects with regard to cervical [P = 0.532] or to ovarian involvement [P = 0.518]. The results linked environmental war pollutants [DU] to alterations in PTEN gene expression in endometrial carcinoma. Furthermore, this finding may explain the overall increasing cancer trends observed in Iraq. Strategies should be considered for the therapeutic targeting of cancers with elevated PTEN gene expression to improve patient outlook.

Keywords: Depleted uranium, PTEN gene, Endometrial carcinoma

Introduction

Endometrial carcinoma is the most common gynecological malignancy in the United States, Japan and other developing countries [1, 2]. Globally it comprises about 3% of all adult female malignancies [3]. There are no reliable studies available to determine incidence of this type of cancer amongst Iraqi women. However, over 380,000 new cases worldwide were diagnosed in 2018 alone [4]. This stresses the urgent need for scientists to tackle such a worldwide problem. There are also many predisposing or risk factors which are believed to play a vital role in the initiation of endometrial carcinoma. These factors may include: diabetes mellitus, hypertension, hormone replacement therapy, obesity and late menopause [4]. Many cases of endometrial carcinoma are sporadic and about 5% have a hereditary predisposition [5]. Endometrial carcinoma is a typical cancer, resulting from numerous genetic errors and alterations. Such alterations are probably caused by faulty repair of DNA damage causing the accumulation of genetic errors.

The most common genetic alteration (30–80%) in endometrial carcinoma [6] occurs in the PTEN gene [7]. PTEN is a tumor suppressor gene located on chromosome 10q23. The protein is involved in different cellular functions such as migration and proliferation [6, 8]. PTEN inactivation is brought about by mutations causing loss of expression and to a lesser extent by loss of heterozygosity [9, 10]. The PTEN protein plays a vital role in controlling the PI3K/AKT pathway by phosphorylating PIP3 at the cell membrane. Loss of functional PTEN protein leads to uninterrupted production of tumorigenic PIP3. MTOR is the major effector of the PI3K/AKT pathway.
pathway, promoting the G1 cell cycle phase and apoptosis-regulating protein interactions [11].

Recent studies have reported that overall cancer incidence has increased at least twofold amongst the Iraqi general population—especially in conflict areas involving Iraqi insurgents and occupation forces. According to the Iraqi Cancer registry, the incidence of cancer increased sharply both after the first and second Gulf war’s [12]. In 1991, the incidence of all cancer types in the Iraqi population was reported to be around 31.05 cases per 100,000 people [12]. However, figures have sharply risen after the war in 2003 to reach a peak of 61.63 cases per 100,000 [12]. Yasseen and co-workers have linked this cancer risk to exposure to DU—a toxic heavy metal with radioactive properties deployed in weaponry [13, 14]. Unfortunately, environmental DU may increase the risk of carcinogenesis in the inhabitants of these polluted war zone areas. More specifically in the female population—DU may impact the pathogenesis of endometrial tumours. We investigated this possibility by quantifying PTEN gene expression profiles and tumor aggressiveness in endometrial carcinoma patients living in either a DU polluted or unpolluted environment.

Main body of text
Materials and methods
Study design and population
The study was carried out at the Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Kufa for 1 year from October 2006 to October 2007. All cases were collected from major hospitals and private laboratories in the middle and south of Iraq. Study ethical approval was obtained from the local Ethics Committee of the University of Kufa, Faculty of Medicine and written consent obtained from each patient. The patients were females, age matched, Arab descend and obtained from the same geographical areas to exclude or minimize the effect of any confounder variables (e.g. age, gender, geographical distribution, ethnicity) on the final outcome. The residual confounder was not an issue as the present work considered all the possible confounding factors during the work, adjusted and controlled accordingly. The cross sectional observational study included 43 cases of endometrial carcinoma. Twenty-one cases came from a conflict region involving DU military activities. A further 22 samples were obtained from endometrial carcinoma patients living in a peaceful area. Only patients who lived in an area which had undergone heavy fighting between the Iraqi insurgents and the occupation forces and remained at the same area for at least 3 years were considered as potentially exposed patients. Those patients labelled as un-exposed lived in areas more than 10 KM from the regional fighting and also had the disease. All cases which were investigated and diagnosed were included. No patient refused to participate in the study, thus, no-response bias affects the final outcome of the work. To avoid any information bias, data was obtained from the electronic database by persons who were blinded to the research questions and outcome.

Normal specimens were also taken from non-malignant hysterectomy (without hyperplasia) for a control as part of standard RTPCR fold change detection.

All samples were formalin-fixed and paraffin embedded tissues. All cases were examined by two independent pathologists to confirm the diagnosis. FIGO system was used as the base for staging and grading of the tumor [15]. Since fixation can effect reliability of downstream RTPCR assays, protocols and timing for formalin fixation of all specimens were kept consistent.

Sample size calculation
A sample size of 32 cases was calculated as the minimal requirement to conduct the present study. The study variable of interest was identified as dichotomous (proportion), and for these sample size calculations the required confidence level and width of the confidence interval was selected. The sample size was calculated using the equation: 

\[ N = 4 \times Z_{\alpha}^2 \times p \times (1 - p)/w^2 \]

where \( Z_{\alpha} \) is the confidence level, \( W \) is the width of the confidence interval and P is the pre-study estimate of the proportion to be measured. 

\[ N = 4 \times 1.96^2 \times 0.8(1 - 0.2)/0.2^2 = 32 \] (required sample size in our study). The proportion of PTEN in endometrial carcinoma is about 80% with a selected confidence interval of ±10 [16].

RNA extraction and PTEN RTPCR gene expression
PTEN gene expression was measured using RNA extracted from formalin-fixed and paraffin embedded tissue. Total RNA was extracted as previously described [17]. PTEN and GAPDH gene probes and primers were obtained from Bioneer company [South Korea]. PTEN gene expression was measured using quantitative real-time PCR. The GAPDH gene was used for normalization of malignant and benign samples of endometrial carcinoma according to the method described by Nolan et al. [17], Fig. 1 qRT-PCR curve. Both target and housekeeping gene data was analyzed using relative quantification gene expression level [fold change] as described by Levak [18]. For each test sample, to generate the relative expression levels, each of the normalized target values (CT values) were divided by the calibrator normalized target value, using the ΔCT method with the GAPDH reference gene.
Statistical analysis
The Qi Square test, exact test and Student’s t-test was used for statistical analysis using SPSS software programs version 23. P value at ≤ 0.05 was used as a level of significance.

Results
PTEN gene expression
The PTEN gene expression mean fold change in the non-exposed group was $0.0031 \pm 0.0029$ and $0.139 \pm 0.185$ in the exposed group, as shown in Table 1. The increase in the PTEN gene expression fold change in patients who lived in the (allegedly exposed) DU areas was significantly higher in comparison with the non-exposed group [$P = 0.001$]. No significant differences were observed in the age distribution between exposed [$56.386 \pm 7.84$] and non-exposed [$55.097 \pm 8.0087$] patients when the age of the subjects was considered [$P = 0.45$].

Tumour stage and grading in relation to gene expression
A total of 8 patients potentially exposed to depleted uranium were diagnosed with a well differentiated grade, 10 moderately differentiated and 3 with poorly differentiated tumors (Table 1). Thirteen patients of the non-exposed cohort were diagnosed with well differentiated tumour grade, 8 of which were moderately differentiated and 1 poorly differentiated. There were no significant differences in gene expression between the different groups when the grade of the tumors was considered [$P = 0.286$].

| PTEN fold change in relation to tumour characteristics in high risk [DU] exposed and non-exposed groups |
|---------------------------------------------------------------------------------------------------------|
| **PTEN fold change**                                                                                      |
| **Group** | **Patient number** | **Mean PTEN** | **Standard deviation** | **Standard error** | **P value** |
| Non-exposed | 22 | 0.0031 | 0.0029 | 0.0006 | 0.001 |
| Exposed | 21 | 0.139 | 0.185 | 0.040 |
| **Age distribution**                                                                                      |
| **Group** | **Patient number** | **Mean age** | **Standard deviation** | **Standard error** | **P value** |
| Non-exposed | 22 | 56.38 | 7.84 | 1.18224 | 0.45 |
| Exposed | 21 | 55.09 | 8.0087 | 1.24076 |
| **Grade differentiation in endometrial carcinoma**                                                        |
| **Group** | **Grade** | **Total** | **P value** |
| Non-exposed | Moderate | 8 | 1 | 13 | 22 | 0.286 |
| Exposed | Poor | 10 | 3 | 8 | 21 |
| Total | Well | 18 | 4 | 21 | 43 |
| **Stage distribution in endometrial carcinoma**                                                           |
| **Group** | **Stage** | **Total** | **P value** |
| Non-exposed | T1 | 11 | 8 | 3 | 22 | 0.98 |
| Exposed | T2 | 10 | 8 | 3 | 21 |
| Total | T3 | 21 | 16 | 6 | 43 |
There were no significant differences in PTEN gene expression fold changes with regard to the stage of the tumor between the exposed and the non-exposed patients \( [P=0.98] \). In non-exposed subjects, 11 cases were of T1, 8 were T2 and 3 cases T3 stage. As for the exposed patients, 10 cases were of T1, 8 were T2 and 3 cases were of T3 stage \( [P=0.98] \).

**Involvement of cervix and ovary**

Table 2 shows that the cervix was involved in five non-exposed cases and in four cases of the exposed patients. The study also found ovarian involvement in 2 of the non-exposed cases and 1 of the exposed group. There were no significant differences found between the two groups with respect to PTEN gene expression for both cervix \( [P=0.532] \) and ovary \( [P=0.518] \).

**Discussion**

DU has been increasingly used as a lethal component of munitions in military conflicts over the last two decades \[19\]. A level of around 320–800 tons of DU was estimated to be used in the first Gulf war in 1991. Nearly the same level was deployed in the 2nd Gulf war in 2003 \[20, 21\]. The carcinogenic effect of DU has been suggested using human samples \[20–27\].

This present study determined significant elevations in PTEN gene expression in endometrial cancer patients lived in areas allegedly polluted with DU when compared to patients in the unpolluted regions (Table 1). This work proves the severity of DNA damage linked to cancer inflicted on the Iraqi civilians by the use of DU-weaponry. This key finding of this study may provide an explanation for the high incidence of cancer in Iraq. This finding is further consolidated by our unpublished observations on the high frequency of sister chromatid exchanges among the Iraqi people who were living in allegedly [DU] exposed areas by comparing them with unexposed control \[28\]. DU may cause nonlethal mutations in critical genes associated with increased proliferation with mutant cells that lead to cancer. Future analysis of the mutational genetic landscape of patient tumours from DU-conflict areas will confirm this.

The present investigation showed that although there was a significant increase in fold change in PTEN gene expression in the high risk [DU] exposure group, other parameters including age, tumour stage and grading, cervix or ovarian involvement had no effect. PTEN gene expression was identified in all stages of tumor. A similar finding was reported by others who also reached the same conclusion \[29–31\]. This observation indicates that PTEN gene alteration is very important for tumour initiation and may synergise with other genetic alterations. Lastly, cervical involvement is an important parameter incorporated in the FIGO staging of endometrial carcinoma \[15\]. However, our work is also in agreement with other investigators who found no correlation between PTEN gene alteration and cervical involvement \[32\].

**Conclusion**

In conclusion, further investigations at the molecular level will be required to clarify the mechanism by which DU may induce or accelerate network signaling pathways involving the PTEN gene linked to endometrial carcinogenesis. Strategies should also be considered and investigated for the therapeutic targeting of cancers with PTEN alterations to improve patient outlook.

| Table 2  Cervical and ovarian involvement of the studied groups |
|----------------------------------|------------------|-----------------|----------------|------------------|
| **Cervical involvement in both high risk [DU] exposed and non-exposed patients** |
| **Group**  | **Cervical involvement**  |                |                |                |
|            | **Free**  | **Involved**  | **Total**  | **P value**  |
| Non-exposed  | 17  | 5  | 22  | 0.532  |
| Exposed  | 17  | 4  | 21  |                |
| Total  | 34  | 9  | 43  |                |

| **Ovarian involvements cross tabulation in high risk [DU] exposed and non-exposed groups** |
|----------------------------------|------------------|-----------------|
| **Group**  | **Ovarian involvement**  |                |
|            | **Free**  | **Involved**  | **Total**  | **P value**  |
| Non-exposed  | 20  | 2  | 22  | 0.518  |
| Exposed  | 20  | 1  | 21  |                |
| Total  | 40  | 3  | 43  |                |
Limitation
Owing to the fact that the PTEN gene is a long gene with a total length of (128,336 bp), thus it is difficult to analyze it fully from formalin fixed paraffin embedded tissue. Accordingly, the present study investigated the whole gene through generating cDNA by isolating total RNA from formalin fixed paraffin embedded tissue.

Abbreviations
PTEN: phosphatase and tensin homolog; DU: depleted uranium; RT-PCR: reverse transcription polymerase chain reaction; PI3 K: phosphatidylinositol-3-kinase; PIP3: phosphatidylinositol 3, 4, 5-trisphosphate.

Acknowledgements
Not applicable.

Authors’ contributions
This work was planned and conducted in collaboration between all authors. ASJ, HAS, EH and AAY contributed to the design of the study and interpreted the data. ASJ, LHM, AAJ and HSA were involved in histopathology examination and examining the patients. EH, MAK, KM and AAJ drafted and critically revised the manuscript. All authors read and approved the final manuscript.

Funding
The authors did not receive any funding for this project.

Availability of data and materials
All the data used in the current study are not publicly available owing to institutional but may be requested on an individual basis from the corresponding author, Professor Akeel Yasseen.

Ethics approval and consent to participate
Study ethical approval was obtained from the local Ethics Committee of the author, Professor Akeel Yasseen.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Kufa, Faculty of Medicine and written consent obtained from each patient.

Received: 3 January 2019 Accepted: 19 October 2019

Published online: 29 October 2019

References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
2. Yamagami W, Nagase S, Takahashi F. Clinical statistics of gynecologic cancers in Japan. J Gynecol Oncol. 2017;28(2):1–13.
3. Martin L, Chang HY. Uncovering the role of genomic ‘dark matter’ in human disease. J Clin Invest. 2012;122(5):1585–99.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
5. Danilidou K, Fragou-Plemenou M, Grammatikakis J, Grigoriou O, Vitoratos N, Kondi-Paftri A. Prognostic significance and diagnostic value of PTEN and p53 expression in endometrial carcinoma. A retrospective clinicopathological and immunohistochemical study. J BUON. 2013;18(1):195–201.
6. Guo C, Song WQ, Sun P, Jin L, Dai HY. LncRNA-GASS induces PTEN expression through inhibiting miR-103 in endometrial cancer cells. J Biomed Sci. 2015;29(22):100.
7. Lee H, Choi HJ, Kang CS, Lee HJ, Lee WS, Park CS. Expression of miRNAs and PTEN in endometrial specimens ranging from histologically normal to hyperplasia and endometrial adenocarcinoma. Mod Pathol. 2012;25(11):1508–15.
8. Shawana S, Kehar SI, Masood S, Aamir I. Immunexpression of Cyclin D1 and PTEN in various endometrial pathologies. J Coll Physicians Surg Pak. 2016;26(4):277–82.
9. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene. 2008;27(41):5497–510.
10. Li L, Ross AH. Why is PTEN an important tumor suppressor? J Cell Biochem. 2007;102(6):1368–74.
11. Djordjevic B, Hennessy BT, Li J. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. Mod Pathol. 2012;25(5):699–708.
12. Ministry of Health, Iraqi Cancer Registry, 2007. https://moh.gov.iq/upload/upfile/ar/166%20%03%20cancer%20registry.pdf. Accessed 4 Sept 2018.
13. Al-Abassi DS, Al-Janabi AA, Al-Toraihi KM, Jabor TA, Yasseen AA. Expression of VEGF in urinary bladder transitional cell carcinoma in an Iraqi population subjected to depleted uranium: an immunohistochemical study. Appl Immunohistochem Mol Morphol. 2009;17(4):307–11.
14. Al-Dujaily EA, Al-Janabi AA, Pierscienek T, Yasseen AA. High prevalence of HER-2/neu overexpression in female breast cancer among an Iraqi population exposed to depleted uranium. J Carcinog. 2008;7:8.
15. FIGO Cancer Report. Cancer of the corpus uteri. Int J Gynecol Obstet. 2015;131(Suppl 2):S96–104.
16. Patra P. Sample size in clinical research, the number we need. Int J Med Sci Public Health. 2012;1(1):5–9.
17. Nolan T, Hands RE, Busin SA. Quantification of miRNA using real-time RT-PCR. Nat Protoc. 2006;1(3):1559–82.
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
19. Bem H, Bou-Rabee F. Environmental and health consequences of depleted uranium use in the 1991 Gulf War. Environ Int. 2004;30(1):123–4.
20. Miller AC, Xu J, Stewart M, Brooks K, Hodge S, Shi L, Page N, McClain D. Observation of radiation-specific damage in human cells exposed to depleted uranium: dicentric frequency and neoplastic transformation as endpoints. Radiat Prot Dosimetry. 2002;99(1–4):275–8.
21. Miller AC, Xu J, Stewart M, McClain D. Suppression of depleted uranium-induced neoplastic transformation of human cells by the phenyl-fatty acid, phenyl acetate: chemoprevention by targeting the p21RAS protein pathway. Radiat Res. 2001;155(1 Pt 2):163–70.
22. Milacic S. Health investigations of depleted-uranium clean-up workers. Med Lav. 2008;99(5):366–70.
23. Milacic S, Simic J. Identification of health risks in workers staying and working on the terrains contaminated with depleted uranium. J Radiat Res. 2009;50(2):213–22.
24. Yang ZH, Fan BX, Lu Y. Malignant transformation of human bronchial epithelial cell (BEAS-2B) induced by depleted uranium. Ai Zhong. 2002;21(9):944–8.
25. Miller AC, Brooks K, Stewart M, Anderson B, Shi L, McClain D, Page N. Genomic instability in human osteoblast cells after exposure to depleted uranium: delayed lethality and micronucleus formation. J Environ Radioact. 2003;64(2–3):247–59.
26. Miller AC, Blakely WF, Livengood D. Transformation of human osteoblast cells by depleted uranium: dicentric frequency and neoplastic transformation as endpoints. Radiat Prot Dosimetry. 2002;99(1–4):275–8.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
28. Al-Saaegh MR, Yasseen AA. Sister chromatid exchanges in human lymphocytes from area of high cancer incidence. M.Sc. Thesis. University of Kufa, Faculty of Medicine; 2009.
29. Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H, Jones MW. Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. Mod Pathol. 2000;13(4):379–88.
30. Salvesen HB, Stefansson I, Kretzschmar E. Significance of PTEN alterations in endometrial carcinoma: a population-based study of mutations, promoter methylation and PTEN protein expression. Int J Oncol. 2004;25(6):1615–23.
31. Akiyama-Abe A, Minaguchi T, Nakamura Y. Loss of PTEN expression is an independent predictor of favorable survival in endometrial carcinomas. Br J Cancer. 2013;109(6):1703–10.
32. Zaino RJ, Abendroth C, Yemelyanova A. Endocervical involvement in endometrial adenocarcinoma is not prognostically significant and the pathologic assessment of the pattern of involvement is not reproducible. Gynecol Oncol. 2013;128(1):83–7.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions