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The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study

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Abstract

Background: Protein kinase B (AKT/PKB) family is frequently amplified in ovarian cancer (OC). To the greatest of our knowledge, there is a lack of published reports about the amplification of the genes belonging to the AKT family among Sudanese women with OC. The present study was conducted to detect the AKT1 gene amplification and its association with tumour types, grades, and ages among Sudanese women with OC, bearing in mind the ethnic variation. **Methods**: This institution-based study included 79 cases of women diagnosed with ovarian cancer (OC) at Omdurman Maternity Hospital in the period 2013-2018. Formalin-fixed, paraffin-embedded (FFPE) tissue sections were used to extract RNA. AKT1 gene amplification was assessed using quantitative real-time PCR. **Results**: The mean age (±SD) of included women was 49.29 (±13.612). The amplification of AKT1 gene was observed in 18/79 (22.8%) of OC women, with a high frequency in women with undifferentiated 1/2 (50%), clear cell 2/6 (33.3%), mucinous 3/11 (27.3%), endometrioid 3/17 (17.6%), and serous carcinomas 5/30 OC (16.7%). High frequency was seen in women with low (26.3%; n = 10/28) rather than in higher (19.5%; n = 8/33) grade carcinoma, and in older (25.8%; n = 8/23) rather than younger (18.2%; n = 2/9) women. No significant association between AKT1 gene amplification and tumour types, grades, and ages of women was observed (Fisher's Exact test: p = 0.405, 0.593 and 0.851, respectively). **Conclusion**: AKT1 gene ampli-

fication arises in around one-fifth of Sudanese women with ovarian cancer (OC). It is seen more in undifferentiated, clear cell, and mucinous tumours types, and more frequently in low tumour grade and older women, but not to a statistically significant level. These outcomes sustenance previous studies suggesting that activated AKT genes have a vital role in OC progression and may offer a plan for targeted therapy and prognostic evaluation.

Keywords

AKT1 Gene Amplification, Ovarian Cancer, Cross-Sectional Study, Quantitative Real-Time PCR, Sudan

1. Introduction

Ovarian cancer (OC) is considered a fatal disease among gynecological cancers. Annually, it marked around 0.2 million ladies all over the world and about 0.125 million deaths [1]. The deadly type of OC is epithelial ovarian cancer (EOC). The majority (>75%) of patients are typically diagnosed in advanced stage [2] as of unfortunate early discovery, recurrence, and further metastasis. For these reasons, the prognosis keeps on poor, the mortality rate is the top, and the five-year survival rate of advanced-stage patients is nearly 30% [3]. Chemotherapy the first-line treatment for OC, calm mainly platinum and taxane does not affect upgraded overall survival [4]. Despite the most patients first respond to cytoreductive surgery and platinum-based chemotherapies; still, several ultimately progress chemo-resistant tumours, relapse, and die [5].

The serine/threonine kinase (AKT), also identified as protein kinase B (PKB), is an oncogenic family of proteins with a molecular weight ~60 kDa [6]. It controls cell growth, survival, proliferation, glycogen metabolism [7], protein synthesis, genome constancy, and apoptosis in response to diverse growth factors and extracellular stimuli [8]. There are three types of AKT, termed PKBα (AKT1), PKBβ (AKT2), and PKBγ (AKT3), and all AKT types are linked with cancer progress [9]. Among them, amplification and over-expression of AKT2 are frequently detected in ovarian cancer (OC) [6]. Akt1 is activated indirectly by insulin and growth factors and is downstream of the phospholipid kinase PI3-K [10]. PDK1 and mTORC2 phosphorylate AKT1 at the threonine 308 and 473 sites, respectively [11] [12], while PIP3 recruits it to the membrane [13]. In cancer, AKT is often activated through a range of mechanisms, comprising amplification of growth factor receptors (e.g., HER2/neu and EGFR) [14], amplification or mutation of phosphatidylinositol 3-kinase (PI3K), and amplification or mutation of AKT isoforms. So, AKT has been widely discovered as an anticancer target therapy [15]. A deeper molecular consideration of tumour biology is necessary to create novel and effective treatment. Therefore, we aimed to study AKT1 gene amplification and its association with tumour types, grades, and ages among Sudanese women with OC, bearing in mind the ethnic variation.

2. Material and Methods

2.1. Samples

This hospital-based, cross-sectional study included 79 adult women diagnosed with ovarian cancer (OC) at Omdurman Maternity Hospital in the period between 2013 and 2018. Additional seven healthy ovarian tissues were used as controls to normalise the results. They were obtained from hospital records of cases diagnosed as unremarkable, which means no histological change. The included cases were adults with histologically confirmed ovarian cancer (inclusion criteria) and no history of another malignancy (exclusion criteria). They were selected conviently. The socio-demographic data were retrieved from the patients' records. The data were collected in the period between 2016 and 2018.

2.2. RNA Extraction and cDNA Preparation

According to the manufacturer's guidelines, total RNA was extracted from the FFPE tissue sections, with the Total RNeasy FFPE kit from Qiagen© (Qiagen, Hilden, Germany). Quanti-Tect Reverse Transcription Kit (Qiagen) was used according to the manufacturer's information to synthesize cDNA. The cDNA has been stored at −20˚C until use.

2.3. Quantitative Real-Time PCR (q-PCR)

AKT1 gene amplification was measured by using quantitative real-time PCR (q-PCR), completed in ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and using the Quanti-TectSYBR Green PCR Kit (Qiagen). DNA was standardized to long interspersed elements (LINE1), which is a repetitive element with comparable copy number for each haploid genome, together in normal DNA samples and cancer cells. Primers were designed by Primer express 3.0 software (Applied Biosystems Foster City CA) [16]. Primers to genomic sequences were (AKT1 F, 5'-ACGGGCACATTAAGATCACA-3'); R, 5-TGCCGCAAAAGGTCTTCATG-3') and (LINE1 F, 5'CCGCTCAACTACATGGAAACTG-3'); R, 5'-GCGTCCCAGAGATTCTGGTATG-3'). The full reaction volume of 20 µL contained 1 µL cDNA, 1 µL forward and reverses primers (Macrogen, Korea), 10 µL of SYBR Green Master Mix, and RNA free water to complete the volume to 20 µL. The subsequent cycling conditions were used: 95˚C for 5 mins; an annealing temperature of 55˚C for 30 s; 50 cycles, and finale with an extension step at 72˚C for 30 s for both AKT1 and LINE1 primers. Nuclease-free water as an alternative of cDNA was incorporated in each run as a negative control. A melting curve was achieved to offer indication for a solitary reaction product. 10 samples were constantly tested (2 times) to verify the reliability of the results. Then the AKT1 gene relative quantification was done via 2-ΔΔCt method [17]. ΔΔCt indicates the variance between the (ΔCt of tumour – average ΔCt of normal tissue), while ΔCt indicates the variance between the (Ct value of the AKT1 gene – the Ct value of LINE1). For gene amplification, Values more than 2.0 were measured positive.

2.4. Statistical Analysis

We use SPSS, version 24 (IBM SPSS) to analysis the results. Descriptive analysis was done for variables (tumor types, grades, and ages of women). To find the statistical significance, fisher's exact test was done between AKT1 gene amplification and tumor types, grades, and ages. p-value < 0.05 was considered to be statistically significant.

3. Results

This study included 79 ovarian cases. Among the 79 cases, the median age was 50 years, and the mean (±SD) was 49.29 (±13.612). All cases were distributed into two age groups: ≤ 50 years (range, 18 - 50 years), 46.8% (n = 37); >50 years (range, 51 - 75 years), 39.2% (n = 31).

Amplification of AKT1 gene was observed in 18/79 OC (22.8%) of women, with a high frequency in women with undifferentiated 1/2 OC (50%), clear cell 2/4 OC (33.3%), others 4/9 OC (30.8%), mucinous 3/8 OC (27.3%), endometrioid 3/14 OC (17.6%), and serous 5/25 OC (16.7%) as seen in **Figure 1**.

High frequency was seen in women with low (26.3%; n = 10/28) rather than in higher (19.5%; n = 8/33) grade carcinomas as seen in **Figure 2**, and in older (25.8%; n = 8/23) rather than younger (18.2%; n = 2/9) women. No significant association between AKT1 gene amplification and tumour histological types, grades, and ages of women was observed (Fisher's Exact test: p = 0.405, 0.593 and 0.851, respectively) as shown in **Table 1**.

4. Discussion

The AKT is an oncogenic protein that regulates cell survival, proliferation, growth, apoptosis, and glycogen metabolism [7]. And its activity is frequently elevated in ovarian cancer [18].

In this study, we assessed the AKT1 gene amplification in Sudanese women with OC. We found AKT1 gene amplification in 22.8% OC, our result disagreed with the results of previous studies described by TCGA analysis (3%) [19] and Despierre *et al.* (53) [20]. Definitely, this study highlighted the lack of consistency with the TCGA analysis and Despierre *et al.*, in part due to difference in sample size, but also ethnic background, the biology of the tumour, and the sensitivity of the methods used.

On the other hand, our study demonstrated AKT1 gene amplification with a relatively high frequency, which reflect the activity of AKT1 gene in OC among Sudanese women and indicating that the AKT1 gene amplification is a common mechanism in the activation of PIK/AKT signaling pathway in Sudanese women with OC.

Accordingly, the AKT has multiple targets [21] and consists of three different isoforms: AKT1-3, which share a high degree of structural similarity and activation [22]. This prompts us to compare our result with AKT2 gene amplification. Our result is higher than Cheng *et al.* study [23], they reported AKT2 gene

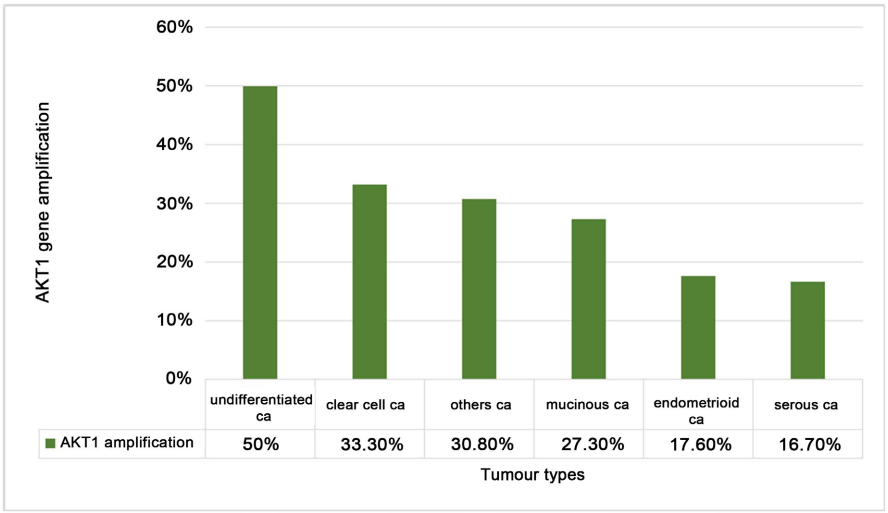

Figure 1. The amplification rate of the v-AKT murine thymoma viral oncogene homologue 1 (AKT1) gene amplification in ovarian cancer according to tumour types among Sudanese women.

Figure 2. The amplification rate of the v-AKT murine thymoma viral oncogene homologue 1 (AKT1) gene in ovarian cancer according to tumour grade among Sudanese women.

Table 1. The association between AKT1 gene amplification status and tumour types, tumour grades, and ages among Sudanese women with ovarian cancer.

	AKT1 gene amplification			p value
	Amplification	No amplification	total	
Tumor types				
Type I	9 (23.7%)	29 (76.3%)	38	0.405
Type II	5 (16.7%)	25 (83.3%)	30	
Total	14	54	68	Missing data = 11
Tumour grades				
High grade	8 (19.5%)	33 (80.5%)	41	0.593
Low grade	10 (26.3%)	28 (73.7%)	38	

Continued

Total	18	61	79	
Tumour ages				
≤50 years	6 (33.3%)	11 (20%)	17	0.335
>50 years	12 (66.7%)	44 (80%)	56	
Total	18	55	73	Missing data = 11
N = 79				

amplification in 13% of primary OC [24]. Regarding the association of AKT1 gene amplification with tumour grades; our study agreed with Nakayama *et al.* study [25] in which amplification of AKT2 has been reported in 18.2% high-grade.

Subsequently, genomic amplification is a common mechanism of oncogene overexpression, this fact encourages us to equate our result with p-AKT overexpression, our result disagrees with Altomare *et al.* their study revealed p-AKT overexpression in 68% OC [25], and Abubaker *et al.* their result identified p-AKT overexpression in 52.1% [16].

The small sample size limited our study. Additional studies with an increased sample size and more additional data (e.g., stage at diagnosis, chemotherapy, radiation) can probably verify the outcome of this study. The impact of combining other genes, which exist in the AKT family, should also be inspected.

5. Conclusion

The current study reveals that AKT1 gene amplification arises in around one-fifth of Sudanese women with OC. It is seen more in undifferentiated, clear cell, and mucinous tumours types, and more frequently in low grade carcinoma and women of older ages, but not to a statistically significant level. In sustenance of lately published literature, our observations foster early reports suggesting that activated AKT gene has a main role in the activation and progression of OC and might offer a novel plan for targeted therapy and prognostic assessment in OC patients.

Ethical Considerations

The study has been approved by the Ethical Boards of Alzaiem Alazhari University (25/1/2017 ع د ك/ أ ز ج/), the Ministry of Health of Khartoum state (22/5/2017أ/44 ط/أع خ/وص/), and Omdurman Maternity Hospital (25/5/2017). patients' informed consent has been waived by the committees, since patients' identity was anonymized, and only laboratory numbers were used.

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Conflicts of Interest

Authors declare that they have no competing interests.

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