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Research Article

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Impacts of microRNA-155 on the Expression of Interleukin-1β and Tumor Suppressor Gene *JADE-1* in Iraqi Women with Cervical Cancer

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Abstract

Background: Cervical cancer poses a significant health challenge globally, with an increase in nations with poor or medium incomes, including Iraq. ***Objective***: This study investigates the molecular interaction between microRNA-155 (miR-155-5p), interleukin-1β (IL-1β), and the tumor suppressor gene *JADE-1*, exploring their roles in the pathogenesis of cervical cancer among Iraqi women. ***Methods***: By analyzing samples from 40 cervical cancer patients and 40 healthy controls, the study investigated the expression levels of miR-155-5p and its impact on IL-1β and *JADE-1* through qRT-PCR and ELISA techniques. ***Results***: The study reveals a significant upregulation of miR-155-5p in patients compared to controls, alongside a notable downregulation of *JADE-1*. While slightly elevating the serum level of IL-1β ($p>0.05$). These changes at the molecular level point to miR-155-5p possibly playing a role in cancer by creating an inflammatory environment around the tumor and decreasing the activity of pathways that stop tumors from growing through *JADE-1*. ***Conclusions***: The study paves the way for further exploration into the mechanistic pathways of these molecules, offering potential biomarkers for early detection, prognosis, and the development of targeted therapies, thus aiming to improve the management and outcomes for women afflicted with cervical cancer in Iraq.

Keywords: Cervical cancer, ELISA, IL-1β, miR-155, *JADE-1*, qRT-PCR.

تأثير microRNA-155 على التعبير عن الإنترلوكين-1بيتا والجين الكابت للورم JADE-1 في النساء العراقيات المصابات بسرطان عنق الرحم

الخلاصة

الخلفية: يشكل سرطان عنق الرحم تحديا صحيا كبيرا على مستوى العالم، مع زيادة في الدول ذات الدخل الفقير أو المتوسط، بما في ذلك العراق. **الهدف**: تبحث هذه الدراسة في التفاعل الجزيئي بين miR-155-5p و IL-1β والجين الكابت للورم *JADE-1* ، واستكشاف أدوارها في التسبب في سرطان عنق الرحم بين النساء العراقيات. **الطرق**: من خلال تحليل عينات من 40 مريضة بسرطان عنق الرحم و 40 انثى من الأصحاء، تم تحليل مستويات التعبير عن miR-155-5p وتأثيره على IL-1β و *JADE-1* من خلال تقنيات qRT-PCR وELISA . **النتائج**: كشفت الدراسة عن زيادة كبيرة في تنظيم miR-155-5p في المرضى مقارنة بالضوابط، إلى جانب انخفاض ملحوظ في تنظيم *JADE-1* مع رفع مستوى مصل IL-1β قليلا. تشير هذه التغييرات على المستوى الجزيئي إلى أن miR-155-5p ربما يلعب دورا في السرطان من خلال خلق بيئة التهابية حول الورم وتقليل نشاط المسارات التي تمنع الأورام من النمو من خلال *JADE-1*. **الاستنتاجات**: تمهد الدراسة الطريق لمزيد من الاستكشاف في المسارات الآلية لهذه الجزيئات، وتقدم مؤشرات حيوية محتملة للكشف المبكر والتشخيص وتطوير العلاجات المستهدفة، وبالتالي تهدف إلى تحسين العلاج للنساء المصابات بسرطان عنق الرحم في العراق.

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INTRODUCTION

Cervical cancer remains a significant public health challenge worldwide, with a disproportionate burden in low- and middle-income countries. Despite global efforts to reduce the incidence of cervical cancer through vaccination and screening programs, many countries, including Iraq, continue to face high rates of morbidity and mortality associated with the disease. The molecular mechanisms responsible for the onset and advancement of cervical cancer are intricate and result from the interaction of many hereditary and environmental elements [1]. Among these, microRNAs (miRNAs) have emerged as key regulators of gene expression, influencing numerous biological processes, including cell proliferation, apoptosis, and carcinogenesis [2]. microRNA-155 (miR-155) is a well-documented oncomiR that has been implicated in the regulation of various cancers, including cervical cancer. Its role in modulating the immune response and inflammation through targets such as interleukin-1β β (IL-1β) has garnered considerable interest, given the critical involvement of inflammation in cancer progression [3]. Its pro-inflammatory properties contribute to the tumor microenvironment by stimulating angiogenesis, enhancing tumor invasiveness, and promoting cancer cell proliferation. IL-1β achieves this by inducing the expression of other pro-inflammatory cytokines, chemokines, and adhesion molecules, thereby recruiting immune cells that can support tumor progression [4]. Moreover, the tumor suppressor gene *JADE-1* (Gene for Apoptosis and Differentiation in Epithelia) is a protein that plays significant roles in cellular processes, including the DNA damage response, chromatin remodeling, and transcription regulation. Its role in cancer has been an area of increasing interest due to its involvement in various cellular mechanisms that can influence tumor progression and suppression [5]. Acts as a tumor suppressor and represents another vital player in the cellular machinery to counteract oncogenesis. Its interaction with miRNAs, particularly miR-155-5P, and the subsequent impact on cervical cancer pathology present an intriguing area of research. miR-155-5p acts as a proinflammatory, oncogenic miRNA across various cancers, promoting proliferation and metastasis by targeting the tumor suppressor gene. Its upregulation correlates with poorer survival in several cancers, indicating its pivotal role in cancer progression [6]. In the context of cervical cancer, the role of the role of miR-155-5p can be explored to understand its influence on the cancer's behavior, particularly in how it may target genes like IL-1 β and *JADE-1*, which are crucial for the inflammatory response and tumor suppression, respectively [7]. Many studies on the exploration of miRNAs in cervical cancer research underscore the intricate relationship between HPV infection, deregulation of cell cycle progression, and cancer development. The modulation of cellular gene expression by HPV E6 and E7 oncoproteins [8], through the alteration of miRNA expression, plays a crucial role in the pathogenesis of cervical cancer [9]. This study aims to elucidate the impacts of miR-155 on the expression of IL-1 β and *JADE-1* in Iraqi women diagnosed with cervical cancer. By focusing on this population, the research seeks to shed light on the specific molecular dynamics within the context of genetic predispositions and environmental influences characteristic of the region. Understanding these relationships is crucial for identifying potential biomarkers for early detection, prognosis, and the development of targeted therapies, offering hope for improved management and outcomes for women afflicted with cervical cancer in Iraq.

METHODS

Study design and patient selection

Forty Iraqi women were diagnosed with cervical cancer through histopathological examination at Baghdad Teaching Hospital, Histopathology Department, Baghdad, Iraq, by approval letter (CSEC/0124/0012). The patients' ages ranged from 45 to 62 years old. The inclusion criteria were Iraqi women with cervical cancer confirmed by pathology without prior surgery, chemotherapy, radiotherapy, or other treatments. Patients who had surgery, radiation therapy, chemotherapy, or were infected, had other malignant tumors, had severe kidney or liver disease, systemic immune diseases, secondary renal hypertension, bone metabolic and pulmonary fibrosis diseases, or had complications from malignant tumors were not eligible. We selected forty healthy volunteers, matched by age with the patient group, as controls.

Sample collection

From each participant, 5 ml of peripheral blood was collected. The blood was divided into two portions: 2.5 ml was placed in EDTA tubes for gene expression analysis, and the remaining 2.5 ml was used for serum separation using gel tubes.

Primers used in this study

The primer sequences used in the laboratory work were designed and manufactured and they were illustrated using a free site: https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool and https://www.mirbase.org. The stem-loop primer for MiR-155 was 5'-GTC GTATCCAGTGCAGGGTCCGAGGTATTCGCACT GGATACGACAACCCC-3', a forward primer was 5'-CGCGCGTTAATGCTAATC-3' and a reverse primer was 5'-CGCTTCACG AATTTGCGTGTCAT-3'. A forward primer for the *JADE-1* gene was 5'-

CGAACTCATGCAGCTCCGA-3' and a reverse primer was 5'-TCCGGATTCAGCTGGTAGGA-3'. The housekeeping gene used in this study was the U6 gene. The forward primer was 5'-GACCTGCTCTGGTGGTCTTG-3', and the and the reverse primer was 5'-GAACCACACTCTGGGACAGG-3'. The primers were designed and used according to Takara Bio Inc., Japan.

Analysis and outcome measurements

The IL-1β levels were determined using an ELISA kit from SungLong Biotechnologies, China. The Direct-zol® RNA Mini Prep Kit (ZYMO Research, USA) was used to extract RNA from EDTA blood samples in accordance with the instructions provided by the manufacturer. In 20 µl of water that was devoid of nucleases, the RNA was extracted. cDNA Synthesis: The Prime Script® RT reagent kit was used for reverse transcription. Ingredients for the reaction were 2µl of 20X prime script reaction buffer, 0.5µl of synthetic stem-loop primer, 4.5 µl of nuclease-free water, and 100 ng/µl of total RNA. After a 15-minute reaction at 42°C in a SaCycler-96 thermal cycler (SACACE, Italy), the sample was heat-inactivated at 85°C for 1 minute and thereafter kept at 4°C. For quantitative polymerase chain reaction (qPCR), 20µl of PCR reaction mixture were made using KAPA SYBR® FAST Universal PCR Master Mix (KAPA, USA). The combination included 10µl of 2X SYBR Green master mix, 4µl of diluted cDNA (1:4), 0.5µl of forward and reverse primer mixes, and 5µl of nuclease-free water. U6 miRNA was used as an internal reference. A melting curve analysis was performed to evaluate the dissociation characteristics of the double-stranded DNA at the end of the qPCR protocol, which included activating the polymerase at 95°C for 7 minutes, 45 cycles of denaturation at 95°C for 10 minutes, and annealing and extension at 60°C for 1 minute.

Ethical Clearance

Scientific studies in Iraq require the Research Ethical Committee's permission before they can proceed. This includes clearance from the University of Baghdad CSEC/0124/0012 and the Ministries of Health.

Statistical analysis

We used SPSS 10.4 statistical software to analyze the data. The mean ± standard error was used to represent the measurement results (SE). The independent sample T-test was used to evaluate the differences in means, with a significance threshold of $P<0.05$. In order to determine the fold change in gene expression, the ΔCT and ΔΔCT methods were used, along with the fold change formula: $2^{\wedge-\Delta\Delta CT}$. The control value of 1 was used as a reference point. Samples with values below 1 were down-regulated, while those above 1 were up-regulated [10].

RESULTS

In the comparative analysis presented in Table 1, serum levels of interleukin-1β (IL-1β) were evaluated between cervical cancer (CC) patients and a control group. The study encompassed equal numbers of participants in each group, with 40 individuals in both the control and CC patient categories.

Table 1: The serum level of IL-1β in CC patients and controls.

Groups	Cases	Age	IL-1 β
Control	40	41.62±1.82	15.272±4.86
CC Patients	40	45.43±2.91	21.342±6.77
p-value		0.421	0.062

Values were expressed as mean±SE.

The mean age of participants was 41.62±1.82 years for the control group and 45.43 ± 2.91 years for the CC patients. The IL-1β level was found to be significantly higher in CC patients, with a mean concentration of 21.342±6.77 pg/mL, compared to the control group (15.272± 4.86 pg/m). Despite the observed difference in IL-1β levels between the two groups, the statistical analysis indicated that the age difference between groups was not statistically significant (p=0.421), nor was the variation in IL-1β levels, although it approached significance (p=0.062). This suggests a trend towards elevated IL-1β levels in CC patients, warranting further investigation to elucidate its potential role in the pathophysiology of cervical cancer (Table 1). Quantitative expression of miR-155-5 and *JADE-1* was confirmed by qRT-PCR, and a relative quantitation method was employed. Gene expression was standardized using a housekeeping gene (U6) and quantified using the folding (2−ΔΔCt) method. A representative RT-qPCR plot is given in Table 2. The gene expression of miR-155-5P in CC patients relative to controls is remarkably upregulated, with a 166.5±8.9-fold increase. This significant upregulation suggests that miR-155-5P plays a crucial role in the molecular landscape of cervical cancer, possibly contributing to tumor development, progression, or the maintenance of malignant phenotypes.

Table 2: Gene expression of miR-155-5P in CC patients and controls

MiR-155-5P	ΔCt	ΔΔCt	2^-DDCT	2^-DDCT Patient/2^-DDCT control
Control	17.1±3.4	0±0.001	3.8±1.1	1±0.01
CC Patient	9.7±2.4	-7.4±1.3	493±12.1	166.5±8.9
p-value	0.01	0.002	0.0000	0.00

Values were expressed as mean±SE.

In contrast, JAD-1 exhibits a notable downregulation in CC patients when compared to the control group, with a fold change of 0.3±0.01. These substantial decreases indicate that JAD-1 may act as a tumor suppressor whose reduced expression is associated with the pathogenesis of cervical cancer (Table 3).

Table 3: The gene expression of *JADE-1* in CC patients and control

JAD-1	ΔCt	ΔΔCt	2^-DDCT	2^-DDCT Patient/2^-DDCT control
Control	9.3±1.4	-4.0±0.2	6.8±1.7	1±0
CC Patient	12.8±2.1	3.5±0.12	0.5±0.01	0.3±0.01
p-value	0.062	0.12	0.001	0.01

Values were expressed as mean±SE.

DISCUSSION

The comparative analysis exploring serum levels of IL-1β and the gene expression profiles of miR-155-5P and *JADE-1* in cervical cancer (CC) patients versus a control group provides insightful observations into the molecular alterations associated with cervical cancer. The observed elevation of IL-1β serum levels in CC patients compared to controls, although not reaching conventional statistical significance (P=0.062), supports previous studies suggesting a pro-inflammatory setting in the tumor microenvironment of cervical cancer. For instance, [11] reported that high IL-1β levels are indicative of an inflammatory response that may facilitate tumor growth and progression by promoting angiogenesis, invasion, and suppression of adaptive immunity. The lack of statistical significance in age differences between groups (p=0.421) further isolates IL-1β elevation as potentially intrinsic to the disease process rather than age-related changes in immune function [12]. The pronounced upregulation of miR-155-5P in CC patients demonstrated that miR-155-5P could modulate the expression of genes involved in cell cycle regulation, DNA repair, and apoptosis mechanisms, thus facilitating oncogenesis. The substantial upregulation observed suggests miR-155-5P could serve as a biomarker for early detection or as a target for therapeutic intervention in cervical cancer [13]. Conversely, the marked downregulation of *JADE-1* in CC patients points to its potential tumor suppressor function. *JADE-1* is known to play roles in cell cycle arrest and apoptosis, and its expression is reduced in CC patients, where loss of *JADE-1* expression is associated with poor prognosis and increased tumor aggressiveness in several cancers [14]. The downregulation of *JADE-1* in CC could therefore indicate a disruption of normal cellular homeostasis mechanisms, contributing to tumorigenesis [15]. *JADE-1* inhibits the growth of cancer stem cells and tumors in pancreatic cancer (PC) via the AKT/mTOR pathway, suggesting that *JADE-1* plays a crucial role in the suppression of cancer development. The AKT/mTOR signaling pathway is known to be a critical regulator of cell growth, proliferation, and survival, and its dysregulation has been implicated in the pathogenesis of various cancers, including pancreatic cancer. By inhibiting this pathway, *JADE-1* can negatively regulate these cellular processes, potentially leading to a suppression of tumor growth and the ability to maintain a non-malignant state [16]. The upregulation of *JADE-1* could, therefore, represent a therapeutic strategy to halt or reverse cancer development. If *JADE-1* expression can be increased in cancer cells, it might counteract the oncogenic signaling pathways that promote tumor growth and survival, effectively suppressing the cancerous phenotype. This approach aligns with the concept of targeting the molecular pathways that cancer cells rely on for their malignant behavior, a strategy that has gained significant traction in cancer therapy development [17,18]. Collectively, these findings underscore the complexity of cervical cancer's molecular landscape, emphasizing the interplay between inflammatory processes, oncogenic miRNAs, and tumor suppressor genes. Future research should aim to elucidate the mechanistic pathways through which IL-1β, miR-155-5P, and *JADE-1* contribute to cervical cancer development and progression. Additionally, investigating these biomarkers' potential in patient stratification, prognosis, and personalized therapy could significantly impact clinical outcomes for cervical cancer patients [19,20].

Conclusion

The study examines the role of microRNA-155 (miR-155-5p) in regulating the expression of IL-1β and *JADE-1* in Iraqi women with cervical cancer. Results show a significant upregulation of miR-155-5p and IL-1β levels in cancer patients, suggesting an oncogenic role. Conversely, a notable downregulation of *JADE-1* in cancer patients suggests its potential as a tumor suppressor gene. This complex interplay highlights the molecular landscape of cervical cancer and suggests miR-155-5p as the potential biomarker for early detection and therapeutic intervention.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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