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Assessment of serum CLEC4M and glutathione levels in newly diagnosed cervical cancer patients in Iraq: A case-control study

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Abstract

Introduction: Cervical cancer is a significant health issue globally, with human papillomavirus (HPV) being a primary causative factor. In Iraq, the prevalence and specific risk factors for cervical cancer remain underexplored, necessitating studies that assess genetic and biochemical markers like C-type lectin domain family four-member M (CLEC4M) and glutathione (GSH).

Objectives: This study was conducted to evaluate serum CLEC4M and GSH Levels in newly diagnosed cervical neoplasm patients in Iraq with the aim of their accuracy evaluation in the diagnosis of this cancer.

Patients and Methods: The case-control study enrolled a total of 70 individuals, comprising 35 recently diagnosed cervical cancer patients and an equal number of 35 healthy female participants who served as the control group. Blood samples were collected from both the patient and control groups to facilitate biochemical analyses. The levels of serum CLEC4M were quantified using the enzyme-linked immunosorbent assay (ELISA) method, while the concentration of GSH was determined using a spectrophotometer. The receiver operating characteristic (ROC) curve analysis was employed to evaluate the sensitivity and specificity of CLEC4M and GSH as biomarkers in diagnosing cervical cancer.

Results: The results indicated that CLEC4M levels were significantly elevated in cervical cancer patients compared to healthy controls; in contrast, GSH levels were significantly lower in the cancer group ($P<0.05$). The ROC curve analysis revealed that CLEC4M exhibited a sensitivity of 71.4% and a specificity of 68.6%, reflecting moderate diagnostic performance for cervical cancer diagnosis; in comparison, GSH demonstrated superior diagnostic capability with a sensitivity of 80% and an exceptional specificity of 97.1%.

Conclusion: This study demonstrates significant differences in biomarker levels associated with cervical cancer, with elevated CLEC4M and decreased GSH levels observed in patients compared to healthy controls. The ROC curve analysis indicates that while CLEC4M has moderate diagnostic performance, GSH shows superior sensitivity and specificity, suggesting its potential as a valuable biomarker for enhancing cervical cancer diagnosis.

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Introduction

Cervical cancer is a prevalent malignant tumor that mostly affects females and is recognized as the second most frequent kind of cancer among women worldwide. This condition is widely acknowledged as a significant threat to women's health. Prolonged infection with the human papillomavirus (HPV) might be regarded as a significant risk factor for the development of cervical cancer (1,2). The incidence and mortality rates of cervical cancer have risen by more than 50% during the last three decades (3).

The C-type lectin domain family four-member M (CLEC4M) is a member of the C-type 55 domain family (4). The CLEC4M cell surface expression facilitates the infection of hepatitis B and C viruses by enhancing the attachment of the virus to the infected cell (5,6). Several studies have shown the role of CLEC4M in a variety of malignancies, including colon cancer. As an example, it has been shown that CLEC4M is associated with worse prognosis in individuals diagnosed with colon cancer (7). Furthermore, this protein is involved in processes such as adhesion, migration, invasion, and the spread of cancer cells to other tissues (8).

Glutathione (GSH) is a tripeptide composed of the amino acids' glycine, cysteine, and glutamine (9). It also contains thiol as a functional group, which serves as a reducing agent (10). GSH, recognized as a prominent antioxidant, exhibits detoxification properties towards electrophilic aggregates and peroxides. This detoxification process is facilitated by the enzymes glutathione S-transferase (GST) and glutathione peroxidase (GPx), which

Key point

The identification of elevated CLEC4M and decreased glutathione (GSH) levels as significant biomarkers highlights the need for integrating these biomarkers into routine diagnostic protocols to improve early detection of cervical cancer. Given that GSH demonstrates superior sensitivity and specificity, it could be prioritized in clinical settings to enhance diagnostic accuracy, potentially leading to earlier interventions and better patient outcomes. Furthermore, these results underscore the necessity for continued research into biomarker development and validation, which can inform future health policies to improve screening strategies. In medical education, incorporating this knowledge into curricula can better prepare healthcare professionals to utilize biomarker data effectively in clinical practice, ultimately fostering a more proactive approach to cervical cancer management.

act as stimulants (11). GSH is a crucial antioxidant with significant implications for humans and microbes (12). Some of the advantages include its ability to detoxify and participate in metabolic processes in glyoxalase, decrease the conversion of ribonucleotides to deoxyribonucleotides, and facilitate protein synthesis by regulating gene expression via thiol: disulfide conversion reactions (13).

The roles of redox and reactive oxygen species (ROS) are very significant in the specific processes of tumor cell migration, tumor cell survival, and tumor cell resistance, which are associated with the progression of cancer (14). Tumor cells exhibit distinct characteristics in regulating ROS homeostasis compared to healthy non-malignant cells (15). Most malignant cells have a higher level of ROS than non-neoplastic cells (16). In several kinds of neoplastic cells, these abundant quantities facilitate their proliferation, reproduction, metastasis, and resilience in multiple microenvironments or locations; a modest to moderate amount of ROS might prioritize genetic instability and tumor eradication (17).

Objectives

The objective of this study is to assess serum levels of CLEC4M and GSH in newly diagnosed cervical cancer patients in Iraq, aiming to evaluate the diagnostic accuracy of these biomarkers in distinguishing cervical neoplasms from healthy controls.

Materials and Methods

Study design and participants

This study employed a case-control design to evaluate serum levels of CLEC4M and GSH in newly diagnosed cervical cancer patients recruited from Al-Amal National Hospital in Iraq. A total of 70 female participants were enrolled, consisting of 35 recently diagnosed cervical cancer patients and 35 healthy female controls matched for age. Blood samples were collected from all participants to analyze the concentrations of serum CLEC4M and GSH. The levels of CLEC4M were quantified using the enzyme-linked immunosorbent assay (ELISA) method, while GSH concentrations were determined using a spectrophotometer. Participants for this study were randomly selected from the Al-Amal National Hospital consultation clinic in Baghdad, Iraq, utilizing a basic random selection method, while healthy volunteers were recruited from the general community. Blood samples were collected from all participants at a single hospital over a time frame extending from October 2022 to February 2023.

Inclusion and exclusion criteria

In this study, the inclusion criteria for participants consisted of female individuals aged 18 years and older, including 35 recently diagnosed cervical cancer patients confirmed by histopathological examination and 35 healthy female volunteers matched for age with no history of cervical cancer or other malignancies. Conversely, exclusion criteria included individuals with a history of any previous malignancies, those who have received treatment for cervical cancer prior to enrollment, participants with significant co-morbidities affecting serum biomarker levels, pregnant or lactating women, and individuals currently taking medications that could influence CLEC4M or GSH levels.

Sample size

The sample size was determined using G*Power version 3.1.9.7 software. The minimum sample size required for this study was determined to be 70 patients. This sample size was calculated based on a power of 90%, a confidence interval of 95% with a two-tailed alpha of 0.05, and an effect size of 0.80. The study had a sample size of 70 participants, consisting of 35 female patients diagnosed with cervical cancer and 35 healthy women who served as controls.

Data collection and laboratory investigation

In this study, written informed consent was obtained from all participants before data collection, and demographic characteristics were recorded. Blood samples, comprising 3 ml from each participant, were collected from both cervical cancer patients and healthy control subjects. These samples were placed in gel tubes and allowed to coagulate at room temperature. Following coagulation, the samples underwent centrifugation at 3000 rpm for five minutes to separate the serum, which was then aliquoted into two Eppendorf tubes and stored at -20°C until further analysis. The parameters assessed included the human CLEC4M, quantified using the ELISA technique, and GSH, measured via spectrophotometry.

Measurement of CLEC4M

The Sandwich ELISA methodology has been used in the quantification of CLEC4M. The capture antibody has been previously immobilized onto 96-well plates. Biotin-conjugated antibodies have been used as detection antibodies. The experiment included the addition of

standards, biotin-conjugated tracking antibodies, and test materials into the wells. Subsequently, a washing buffer was used to cleanse the wells. HRP-streptavidin was introduced, followed by the removal of unbound conjugates by the use of a washing buffer. The TMB (3,3A,5,5A-tetramethylbenzidine) substrates have been used to predict the outcome of the horseradish peroxidase enzymatic reaction. The TMB substrate has been activated by the application of horseradish peroxidase to produce the desired blue colour, which then changes to yellow with the addition of an acidic stabilizing solution. The density of the yellow amount has been shown to correlate with the quantity of the sample that has been collected on the plate. The optical density (OD) absorbance is measured at a wavelength of 450nm using a microplate reader, and afterwards, the corresponding concentration may be determined (18).

Determination of serum GSH concentration

The serum GSH concentration was determined using a spectrophotometer (19).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 27 (IBM Corp, USA). To evaluate the differences in biomarker levels between the case and control groups, descriptive statistics, including means and standard deviations, were calculated for both groups. The significance of differences in serum CLEC4M and GSH levels was assessed using the independent t-test, with a significance level set at $P < 0.05$. To evaluate the diagnostic performance of CLEC4M and GSH, the receiver operating characteristic (ROC) curve analysis was conducted to determine sensitivity and specificity values for each biomarker. The area under the ROC curve (AUC) was also calculated to provide an overall measure of diagnostic accuracy. Results were interpreted within the context of their clinical relevance, particularly concerning their potential utility as biomarkers in the diagnosis of cervical cancer.

Results

The analysis of pathological samples reveals various characteristics related to cancer type and stage in Table 1. Among the cancer types, adenocarcinoma was the most prevalent, followed by squamous cell carcinoma and adenosquamous carcinomas. In terms of cancer staging for adenocarcinoma, a range of stages was represented, with notable frequencies observed in stages 1A2 and 2A. For squamous cell carcinoma, stage 2A also had a significant presence, while other stages such as 1A1 and 1B2 were less common. Adenosquamous carcinomas were limited to early stages, with equal representation in stages 1A1 and 1A2 (Table 1).

Table 2 provides a comparative analysis of key parameters between the case group of cervical cancer patients and the healthy control group. The mean age of participants in the case group was higher than that of the control group, although this difference was not statistically significant. Regarding CLEC4M levels, the case group exhibited elevated concentrations compared to the controls, with a statistically significant difference noted. Conversely, the levels of GSH were significantly lower in the case group compared to the control group, indicating a notable disparity in antioxidant status between cervical cancer patients and healthy individuals (Table 2).

The results indicated that CLEC4M levels in cervical cancer patients were significantly greater than those in healthy individuals; in comparison, the levels of GSH were notably lower in the cancer patient group (Figures 1 and 2).

Table 1. The pathological characteristics of cervical cancer samples of patients in the case group

Characteristics of pathological samples			Frequency	Percent
Cancer type		Adenocarcinoma	19	54.28
		Squamous cell carcinoma	14	40
		Adenosquamous carcinomas	2	5.72
Cancer stage	Adenocarcinoma (n = 19)	1A1	3	15.8
		1A2	5	26.3
		2A	4	21.1
		1B1	2	10.5
		1B2	2	10.5
		2B	2	10.5
		3B	1	5.3
	Squamous cell carcinoma (n = 14)	2A	4	28.6
		1A1	1	7.1
		1A2	1	7.1
		4A	1	7.1
		1B1	1	7.1
		1B2	2	14.4
		2B	4	28.6
	Adenosquamous carcinomas (n = 2)	1A1	1	50
		1A2	1	50

Table 2. Comparative analysis of age, CLEC4M, and GSH levels between cervical cancer patients in the case group and healthy individuals in control groups

Parameters	Group				*P* value
	Case (Cervical cancer patients, n = 35)		Control (Healthy individuals, n = 35)		
	Mean	SD	Mean	SD	
Age (year)	51.34	8.18	45.54	11.65	0.124*
CLECM4 (ng/mL)	128.746	48.02	100.320	47.51	0.015*
GSH (mg/L)	816.70	495.91	1209.2	219.23	<0.001*

SD, Standard deviation. * Independent *t* test.

The ROC curve analysis demonstrated that CLEC4M concentrations in the diagnosis of cervical cancer exhibited a sensitivity of 71.4% and a specificity of 68.6%, indicating moderate diagnostic performance, while GSH levels showed a higher sensitivity of 80% and an impressive specificity of 97.1%, suggesting that GSH may serve as a more reliable biomarker for distinguishing between cervical cancer patients and healthy individuals (Figure 3).

Discussion

The findings of this study provide significant insights into the diagnostic potential of serum CLEC4M and GSH levels in cervical cancer patients. The results demonstrated that CLEC4M levels were significantly elevated in the cancer group compared to healthy controls, while GSH levels were notably lower, with both differences achieving statistical significance. These alterations in biomarker levels suggest a potential role for CLEC4M as a tumor-associated marker, which aligns with existing literature indicating its involvement in various malignancies. The presence of elevated levels of CLIC4M in the bloodstream has been reported as a potentially significant indicator for the development of cancer during its early phases (19). Several studies have shown a significant association between the interdependent role of CLEC4M and tumor progression. As demonstrated by previous research (20), there is an established association between the CLEC4M gene and the development of tumor growth (21). Furthermore, a number of studies have indicated that CLEC4M may promote the progression of metastases (22,23). Meng et al revealed that the overexpression of CLEC4M effectively suppresses proliferation in hepatocellular carcinoma

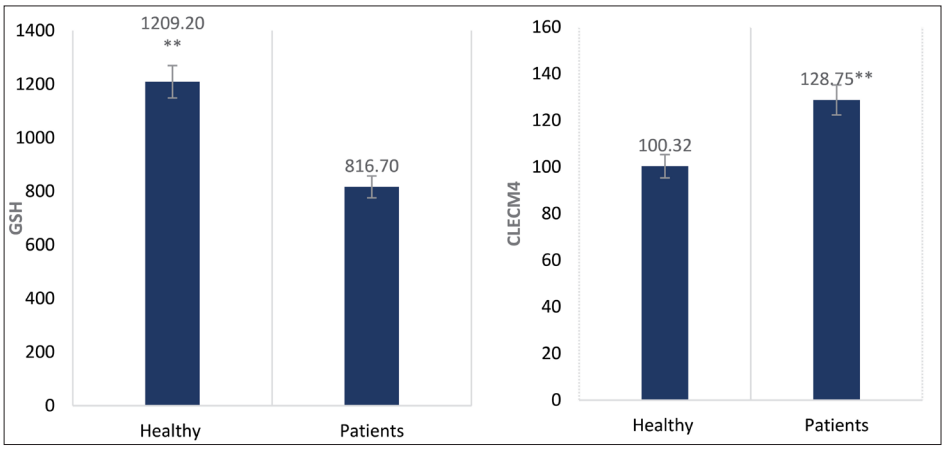

Figure 1. The comparison of CLECM4 and GSH concentrations in cervical cancer patients and healthy individuals.

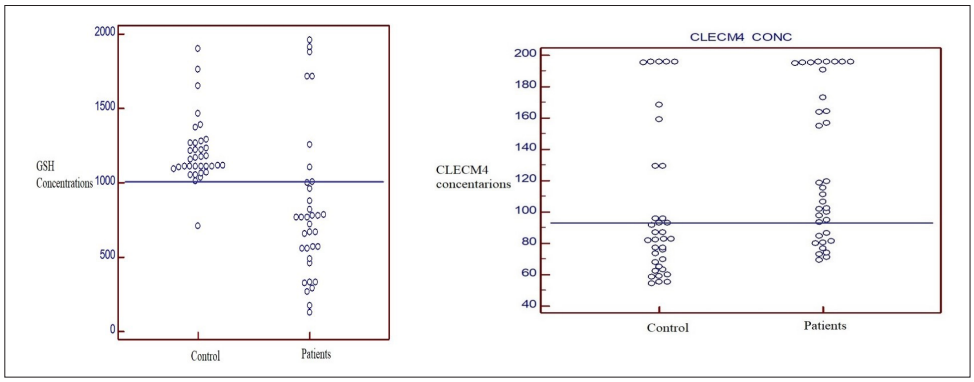

Figure 2. The comparison of CLECM4 and GSH concentrations in cervical cancer patients and healthy individuals using the interactive dot diagram.

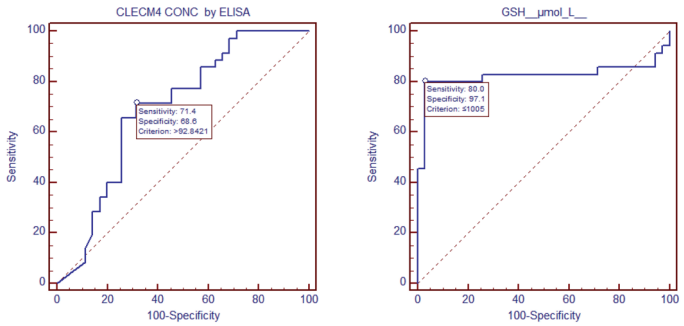

Figure 3. The diagnostic accuracy of CLECM4 and GSH concentrations in cervical cancer diagnosis using ROC curve analysis.

(HCC) cell lines and enhances cell death, which means documenting a novel tumor-suppressive mechanism of CLEC4M in hepatocellular carcinoma (24). Previous studies on gastrointestinal tract cancer have shown that the concentration of CLEC4M is elevated in patients with gastric cancer, especially in cases where there is liver metastasis (25). Additionally, the levels of CLEC4M are strongly associated with the advanced stage of the disease; these findings demonstrate a distinct role of CLEC4M in the process of tumor metastasis (26). A prior investigation has shown that individuals diagnosed with lung cancer had elevated levels of CLIC4M in their bloodstream compared to healthy individuals, who had much lower levels of this indicator (27). The CLEC4M (C type) receptor is mostly localized on the endothelial cells of several organs, the receptor has previously been recognized as a binding site for critical components, particularly viruses (28). Recently, there has been an assessment of the scientific value of CLEC4M in the context of cancer. An increase in CLEC4M concentrations has been suggested as a potentially helpful measure for the early detection of colon cancer (22).

The ROC curve analysis elucidated the diagnostic capabilities of these biomarkers, revealing that CLEC4M exhibited a sensitivity of 71.4% and specificity of 68.6%, indicating moderate diagnostic performance for cervical cancer. In contrast, GSH displayed superior diagnostic accuracy with a sensitivity of 80% and an exceptional specificity of 97.1%. This suggests that GSH may serve as a more reliable biomarker for distinguishing cervical cancer patients from healthy individuals. The process involves the meticulous identification and integration of diverse viral surface glycoproteins, which include high mannose N-linked oligosaccharides, in a way that is reliant on calcium (20).

Previous studies have shown a potential association between HPV and cervical cancer (29,30). Furthermore, it has been reported that HPV is present in 99.7% of cervical cancer cases (31). According to recent research that examined over 150 000 images with normal cervical cytology, the worldwide prevalence of HPV is around 10% (32).

Squamous cell carcinoma is widely recognized as the predominant form of cervical cancer, accounting for around 70% of cases. The incidence of aggressive cervical adenocarcinoma and its variants has steadily increased in recent years. This kind of neoplasia accounts for roughly 25% of all newly diagnosed invasive cervical malignancies (33). HPV infections are often recognized temporarily, although they may lead to cytological abnormalities that are typically reversible. The transmission of this virus occurs by direct contact with the epidermis and may result in transmission to another individual even in the absence of observable symptoms (34,35). One potential result that might be anticipated is the development and progression of neoplasia (36). In addition to other viral receptors and maybe papilloma viruses (37), recent studies have shown a correlation between CLEC4M and carcinogenesis (22). The blood CLEC4M levels exhibited a higher magnitude in individuals diagnosed with colon cancer compared to those in the healthy control group (38).

In the current study, it was found that GSH levels decreased in cervical cancer patients when compared with the healthy group; this finding in agreement with several previous studies (40,41).

It is known that GSH is transformed into GSH diphosphate, and during reduction it returns to being GSH, which is considered an important antioxidant that combats the occurrence of the oxidation process in the body and rids the body of toxic influences and factors inside the cells, which reduces the chances of cancer cells forming. It works as a secondary antioxidant and protects against many factors. Cytotoxic and carcinogenic by eliminating ROS (40).

Elevated amounts of GSH have been seen in several cancer types (20). The GSH molecule plays a significant function in several physiological activities, such as generating (39,40). The genesis and spread of cancer are often attributed to oxidative stress processes, which are seen as significant catalysts (7). It is commonly believed that the administration of antioxidants as a therapy may provide protection against cancer (41). One of the crucial processes in the human body involves oxidation-reduction reactions, which play a significant role in maintaining cellular equilibrium (42). These reactions include the participation of GSH, which serves to protect cells from oxidative damage. Additionally, it plays a significant role

in several metabolic processes (43). Elevated levels of GSH have been seen in several cells, both benign and malignant, and have been shown to be associated with a stimulated response and essential for the progression of the cell cycle (44). Elevated GSH concentrations were shown to be significantly associated with a poor prognosis in cancer patients, independent of other factors. GSH levels may serve as a predictive indicator (45).

Overall, these results underscore the importance of evaluating both CLEC4M and GSH as potential biomarkers in the clinical setting for cervical cancer diagnosis. The significant differences in their serum levels highlight their possible roles in the pathophysiology of cervical cancer and their utility in enhancing diagnostic accuracy. However, it is essential to acknowledge the limitations of this study, including the relatively small sample size and the need for further validation in larger cohorts to confirm these findings. Future research should focus on exploring the underlying mechanisms linking these biomarkers to cervical cancer progression and their potential roles in patient management and treatment strategies. In conclusion, this study contributes valuable data to the growing body of evidence supporting the use of serum biomarkers in the early detection and diagnosis of cervical cancer, particularly within the Iraqi population.

Conclusion

In conclusion, the findings of this study highlight the significant alterations in biomarker levels associated with cervical cancer, specifically noting elevated CLEC4M concentrations and reduced GSH levels in patients compared to healthy controls, with statistical significance established. The ROC curve analysis further underscores the diagnostic potential of these biomarkers, revealing that while CLEC4M exhibits moderate sensitivity and specificity for cervical cancer diagnosis, GSH demonstrates superior diagnostic capability with higher sensitivity and exceptional specificity. These results suggest that both CLEC4M and GSH could serve as valuable biomarkers in the clinical assessment of cervical cancer, with GSH particularly standing out as a promising candidate for enhancing diagnostic accuracy and patient stratification. Future studies should focus on validating these findings in larger cohorts to further elucidate the clinical utility of these biomarkers in cervical cancer management.

Limitations of the study

The study has several limitations that should be acknowledged. First, the relatively small sample size of 70 individuals, consisting of only 35 cervical cancer patients and 35 healthy controls, may limit the statistical power and generalizability of the findings to the broader population. Additionally, the case-control design does not account for potential confounding variables such as lifestyle factors, comorbidities, and environmental influences that could affect biomarker levels. Furthermore, the cross-sectional nature of the study restricts the ability to establish causal relationships between biomarker levels and cervical cancer progression. Lastly, while the study provides valuable insights into the diagnostic potential of CLEC4M and GSH, further research with larger cohorts and longitudinal designs is necessary to validate these findings and assess their clinical applicability in diverse populations.

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Authors' contribution

Conceptualization: Huda Jaber Waheed.
Data curation: Huda Jaber Waheed.
Formal analysis: Huda Jaber Waheed.
Investigation: Wassan Abdul Kareem Abbas.
Methodology: Wassan Abdul Kareem Abbas.
Project management: Huda Jaber Waheed.
Resources: All authors.
Supervision: Wassan Abdul Kareem Abbas.
Validation: Wassan Abdul Kareem Abbas.
Writing—original draft: All authors.
Writing—reviewing and editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

The research was conducted in accordance with the principles outlined in the Declaration of Helsinki. This study resulted from the research approved by the Ethic Research Committee of Pharmacy College (Approval No. 49), Mustansiriyah University, Baghdad, Iraq. Written informed consent was obtained from each participant. Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

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