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THE ROLE OF SERUM ITF, IBA-1, AND CALGRANULIN B LEVELS IN THE EARLY DIAGNOSIS AND PROGNOSIS OF CERVICAL CANCER

ULOGA NIVOA SERUMSKIH ITF, IBA-1 I KALGRANULINA B U RANOJ DIJAGNOZI I PROGNOZI KOD KARCINOMA CERVIKSA

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Summary

Background: To explore the clinical value of serum Intestinal Trefoil Factor (ITF), Ionized Calcium-Binding Adapter Molecule 1 (IBA-1), and Calgranulin B levels in the early auxiliary diagnosis and prognostic evaluation of cervical cancer.
Methods: The cervical cancer group consisted of 256 patients who received a cervical cancer diagnosis at this hospital between January 2023 and June 2024. One hundred fifty people who were diagnosed with cervical intraepithelial neoplasia (CIN) at this hospital within the same time period made up the CIN group. Ninety women who underwent health checks at this facility during the same time period comprised the healthy control group. The diagnostic effectiveness of serum ITF, IBA-1, and Calgranulin B levels in patients with cervical cancer, as well as their correlations with clinical indicators and prognosis, were examined, along with changes in these levels across groups.
Results: The cervical cancer group's blood ITF, IBA-1, and Calgranulin B levels were significantly higher than those of the CIN and healthy control groups. Compared to the healthy control group, the CIN group's serum ITF, IBA-1, and Calgranulin B levels were considerably higher ($P<0.05$). In diagnosing cervical cancer, serum levels of ITF, IBA-1, and Calgranulin B are highly valuable. When all three signs were detected together, the sensitivity was 89.4%. The specificity

Kratak sadržaj

Uvod: Clj je bio da se ispita klinička vrednost seruma intestinalnog trefoil faktora (ITF), jonizovanog kalcijum-vezujućeg adapter proteina 1 (IBA-1) i Kalgranulina B u ranoj pomoćnoj dijagnozi i proceni prognoze kod karcinoma cerviksa.
Metode: Grupu sa karcinomom cerviksa činilo je 256 pacijentkinja koje su dobile dijagnozu u ovoj bolnici od januara 2023. do juna 2024. godine. Grupu sa cervikalnom intraepitelnom neoplazijom (CIN) činilo je 150 osoba gde je dijagnoza postavljena u istom periodu. Kontrolnu grupu je činilo 90 žena koje su u istom periodu obavljale sistematske preglede. Ispitana je dijagnostička efikasnost seruma ITF, IBA-1 i Kalgranulina B kod pacijentkinja sa karcinomom cerviksa, njihova korelacija sa kliničkim indikatorima i prognozom, kao i promene ovih nivoa između grupa.
Rezultati: Nivoi ITF, IBA-1 i Kalgranulina B u serumu grupe sa karcinomom cerviksa bili su značajno viši nego u grupama sa CIN i zdravim kontrolama. U poređenju sa zdravim kontrolama, nivoi seruma u CIN grupi bili su značajno viši ($P<0,05$). U dijagnostikovanju karcinoma cerviksa, nivoi seruma ITF, IBA-1 i Kalgranulina B imaju visoku vrednost. Kada su svi tri markera detektovana istovremeno, osetljivost je iznosila 89,4%, specifičnost 97,6%, a površina ispod ROC krive (AUC) bila je 0,961,

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was 97.6%, and 0.961 was the receiver operating characteristic curve's area under the curve, which was significantly greater than that of ITF (Z=4.620, P<0.05), IBA-1 (Z= 4.167, P<0.05), and Calgranulin B (Z=5.210, P<0.05) alone in independent testing. Serum ITF, IBA-1, and Calgranulin B levels were significantly higher in patients with poorly differentiated, clinical stage II, HPV-positive cervical cancer with lymph node metastases than in patients with moderately differentiated, clinical stage I, HPV-negative cervical cancer without lymph node metastases. These differences were statistically significant (P<0.05). However, there were no statistically significant differences in serum ITF, IBA-1, or Calgranulin B levels among cervical cancer patients of different ages, pathological types, maximum tumour diameters, depths of myometrium invasion, vascular invasion, or nerve invasion (P>0.05). Serum ITF, IBA-1, and Calgranulin B levels in the nonsurviving group were substantially higher than those in the surviving group at the 1-year follow-up (P<0.05).

Conclusions: The levels of serum ITF, IBA-1, and Calgranulin B have high clinical value in the diagnosis and prognostic evaluation of cervical cancer. The combined detection of these three indicators can improve the auxiliary diagnostic efficacy of cervical cancer.

Keywords: intestinal trefoil factor, calcium-binding adapter molecule 1, Calgranulin B, cervical cancer, prognostic analysis

Zaključak: Nivoi seruma ITF, IBA-1 i Kalgranulina B imaju visoku kliničku vrednost u dijagnostici i proceni prognoze kod karcinoma cerviksa. Kombinovana detekcija ovih triju markera može poboljšati pomoćnu dijagnostičku efikasnost karcinoma cerviksa.

Ključne reči: intestinalni trefoil faktor, kalcijum-vezujući adapprotein 1, Kalgranulin B, karcinom cerviksa, prognostička analiza

Introduction

After breast cancer, cervical cancer is the second most prevalent malignant tumour in women (1). Its incidence rate is increasing annually, and approximately half of patients die, which poses a serious threat to women's health and survival. Owing to the lack of specific markers for cervical cancer, early diagnosis and prognosis assessment indicators remain the focus of scholars' research. In recent years, serological markers have gradually been recognized by scholars (2). They offer the advantages of repeatability, rapidity, convenience, and noninvasiveness, and have attracted widespread attention from scholars (3). Intestinal Trefoil Factor (ITF) is a polypeptide family of trefoil structures that is highly expressed in malignant tumours such as bladder cancer and gastric cancer. It promotes tumour invasion and inhibits apoptosis (4). It has been confirmed in cervical cancer cells and tissue specimens that ITF is highly expressed and participates in the occurrence and development of cervical cancer. Ionized Calcium-Binding Adapter Molecule 1 (IBA-1) is a cytokine closely related to cellular immune regulation. It was initially used for immune monitoring (5). Later, it was found to be highly expressed in cervical cancer and was associated with the lymph node metastasis of cervical cancer. Calgranulin B is an essential member of the family of serum markers (6). It is upregulated in cervical cancer and is closely related to the invasion, metastasis, and proliferation of cervical cancer (7).

Although inevitable progress has been made in the early detection and intervention of cervical cancer with the advancement of screening technology, the early diagnosis of cervical cancer still faces challenges owing to the limited sensitivity and specificity of existing markers (8). ITF, IBA-1, and Calgranulin B are biomarkers that have attracted increasing attention in tumour research in recent years, and their roles in tumour cell growth, metastasis, and the immune response have been revealed (9). Previous studies have shown that ITF, IBA-1, and Calgranulin B are closely associated with tumour occurrence, development, and metastasis across various tumour types. Still, their specific roles and clinical applications in cervical cancer remain unclear (10–12).

Therefore, exploring the potential of these biomarkers in the early diagnosis and prognosis assessment of cervical cancer not only helps enrich the biomarker system of cervical cancer but also provides new diagnostic and predictive tools for clinical practice, promoting the realization of early detection and personalized treatment of cervical cancer.

Materials and Methods

General information

An average age of 39.75±8.20 years was found among 256 patients between the ages of 28 and 52, who were chosen as the cervical cancer group, those who got a cervical cancer diagnosis at our hospital between January 2023 and June 2024. Pathology revealed that 164 cases were moderately differentiated, and 92 cases were poorly differentiated.

The pathological types were as follows: The human papillomavirus (HPV) test yielded 204 positive cases and 52 negative cases. Clinical stages: Stage IA patients were 14, IB patients were 112, IIA patients were 66, and IIB patients were 64. For the CIN group, 150 patients aged 29–52 who received a cervical intraepithelial neoplasia (CIN) diagnosis at our hospital during that time were selected. Their average age was 39.10±9.20 years.

The healthy control group consisted of 90 women who visited our hospital for health examinations during the same time period. These patients were aged 30–59 years, with an average age of 38.95±8.12 years. All subjects provided informed consent, signed the informed consent form, and this study was reviewed and approved by the ethics committee of our hospital.

Inclusion criteria: (1) Both cervical cancer and CIN were confirmed by pathological examination; (2) Age: 25 to 59 years old; (3) No radiotherapy, chemotherapy, or immunotherapy was received before enrolment.

Exclusion criteria: (1) Malignant tumours of other systems; (2) Other diseases of the female reproductive system; (3) Pregnant and lactating women; (4) Haematological and immune diseases; (5) Dysfunction of vital organs such as the heart, liver, and kidneys; (6) Intellectual decline and mental illness.

Treatment methods

Extensive hysterectomy with pelvic lymph node dissection was performed in patients with Stage IB to Stage IIB cervical carcinoma, while patients with stage IA cervical cancer underwent subextensive hysterectomy. Conventional intracavitary brachytherapy was administered weekly for two sessions before surgery in patients with Stage IB to IIB cervical cancer and a maximum cervical tumour diameter >4 cm. Neoadjuvant chemotherapy was then administered (specify regimen). Surgical treatment was performed 2 weeks after the completion of radiotherapy. Postoperative radiotherapy and chemotherapy were indicated in patients whose pathological examination revealed any of the following: tumour invasion of ≥1/2 of the cervical muscular layer, tumour involvement at the resection margin, pelvic lymph node metastasis, or vascular invasion.

Blood sample preservation and index detection

When each group of subjects was enrolled, 5 mL of fasting elbow venous blood was drawn and placed in an anticoagulant tube. The blood was centrifuged for ten minutes at 3,000 rpm. ITF, IBA-1, and Calgranulin B serum levels were measured by placing the supernatant in a refrigerator set to -70 °C. The levels of serum ITF, IBA-1, and Calgranulin B were measured by enzyme-linked immunosorbent assay. The kits were purchased from Shanghai Lianmai Bioengineering Co., Ltd.

Observation indicators

Changes in the serum ITF, IBA-1, and Calgranulin B levels in each group were observed, as were the effects on the serum ITF, IBA-1, and Calgranulin B levels in the auxiliary diagnosis of cervical cancer.

Statistical processing methods

The statistical program SPSS 20.0 was used for data processing and analysis. $\bar{x}\pm s$ is the expression for measurement data that follows a normal distribution. Two groups were compared using the t-test, one-way analysis of variance was used for comparisons among multiple groups, and the least significant difference (LSD) test was used for pairwise comparisons among multiple groups. Multivariate logistic regression analysis was conducted on the occurrence of cervical cancer, with CIN as the control. The diagnostic effectiveness of serum ITF, IBA-1, and Calgranulin B levels was examined using receiver operating characteristic (ROC) curves.

Results

Comparison of serum ITF, IBA-1, and Calgranulin B levels among the healthy control group, CIN group, and cervical cancer group

The levels of serum ITF, IBA-1, and Calgranulin B in the cervical cancer group were significantly higher than those in the CIN and healthy control groups. Serum levels of ITF, IBA-1, and Calgranulin B were considerably higher in the CIN group than in the healthy control group ($P<0.05$), see *Table I*.

Diagnostic efficacy of serum ITF, IBA-1, and Calgranulin B levels in cervical cancer

With CIN as the control, multivariate logistic regression analysis was conducted on the three indicators based on the presence of cervical cancer, and $Y=0.27\times XITF+0.02\times XIBA\text{-}1+0.03\times XCalgranulin\ B\text{-}26.06$ was obtained as the combined detection index. The sensitivity of the combined detection of the three indicators was 89.4%, the specificity was 97.6%, and the area under the curve (AUC) was 0.961, which was significantly greater than that of ITF ($Z=4.620$, $P<0.05$), IBA-1 ($Z=4.167$, $P<0.05$), and Calgranulin B ($Z=5.210$, $P<0.05$), which were detected separately, see *Table II*.

Table I Comparison of serum ITF, IBA-1, and Calgranulin B levels among the healthy control group, CIN group, and cervical cancer group.

Group	n	ITF (μg/L)	IBA-1 (pg/mL)	Calgranulin B (ng/mL)
Healthy control group	90	14.45±3.20	78.30±19.35	83.20±23.49
CIN group	150	57.77±5.03	189.22±64.18	152.32±47.15
Cervical cancer group	256	67.21±7.19	292.78±63.39	223.29±51.45
F		1538.267	291.349	199.539
P		<0.001	<0.001	<0.001

Table II Diagnostic efficacy of serum ITF, IBA-1, and Calgranulin B levels in cervical cancer.

Indicator	Truncation value	Sensitivity (%)	Specificity (%)	AUC	95% CI
ITF	63.63 pg/L	71.4	89.7	0.860	0.815~0.913
IBA-1	251.98 pg/mL	77.6	84.3	0.878	0.825~0.910
SI00-All	159.04 ng/mL	89.4	65.6	0.847	0.789~0.894
3 Joint Projects	–	89.4	97.6	0.961	0.937~0.981

Table III Comparison of serum ITF, IBA-1, and Calgranulin B levels in cervical cancer patients with different clinical indicators.

Clinical indicators	n	ITF (μg/L)	t	P	IBA-1 (pg/mL)	t	P	Calgranulin B (ng/mL)	t	P
Age (years)			0.823	0.417		0.842	0.391		0.794	0.433
≥40	72	66.47±6.80			285.17±63.69			217.54±51.30		
<40	184	67.63±7.21			295.76±63.37			225.55±51.58		
Pathological differentiation			13.545	<0.001		14.851	<0.001		14.069	<0.001
Poorly differentiated	92	74.64±4.68			357.02±30.26			275.32±28.67		
High school differentiation	164	63.19±6.68			256.69±46.05			194.05±35.83		
Pathological type			0.697	0.482		0.763	0.442		0.737	0.467
Adenocarcinoma	94	66.73±7.24			287.18±66.08			218.81±53.85		
Squamous cell carcinoma	162	67.64±7.18			296.03±61.97			225.84±50.17		
Maximum diameter of tumour (cm)			0.092	0.925		0.136	0.897		0.127	0.904
≥4	108	67.23±7.08			291.81±64.21			222.63±52.40		
<4	148	67.36±7.21			293.32±63.15			223.78±51.03		

Depth of infiltration into the muscle layer			0.497	0.625		0.355	0.728		0.331	0.739
≥1/2	126	67.52±7.11			294.79±64.26			224.86±52.84		
<1/2	130	66.90±7.11			290.83±62.98			221.78±50.44		
HPV			10.162	<0.001		11.564	<0.001		15.107	<0.001
Positive	204	69.62±5.65			315.64±45.78			241.70±38.42		
Negative	52	57.72±3.97			203.09±37.98			150.69±23.88		
Clinical Staging			13.601	<0.001		14.800	<0.001		14.644	<0.001
Phase I	126	61.62±4.35			241.69±42.04			181.91±32.15		
Phase II	130	72.62±4.84			342.20±34.64			263.21±30.74		
Vascular invasion			0.408	0.680		0.585	0.565		0.480	0.620
Yes	34	66.65±7.41			284.43±64.88			217.52±55.26		
No	222	67.31±7.17			294.06±63.36			224.16±51.05		
Lymph node metastasis			12.231	<0.001		15.042	<0.001		13.868	<0.001
Yes	52	77.62±3.54			377.05±24.96			293.48±25.49		
No	204	64.65±5.17			271.20±50.92			205.30±39.69		
Neural infiltration			1.552	0.124		1.821	0.073		1.660	0.091
Yes	44	65.15±7.92			270.42±72.97			206.78±59.12		
No	212	67.75±6.96			297.30±60.58			226.62±49.21		

Table IV Comparison of serum ITF, IBA-1, and Calgranulin B levels between the death group and the survival group.

Group	n	ITF (μg/L)	IBA-1 (pg/mL)	Calgranulin B (ng/mL)
Survival group	176	63.51±4.78	261.04±47.28	197.38±36.73
Death group	80	75.43±4.29	362.52±28.50	280.20±27.53
t		13.442	15.016	14.163
P		<0.001	<0.001	<0.001

Comparison of serum ITF, IBA-1, and Calgranulin B levels in patients with different clinical indicators

Compared to patients with moderately differentiated cervical carcinoma, those with poorly differentiated, HPV-positive, stage II clinical, and lymph node metastases had significantly higher serum levels of ITF, IBA-1, and Calgranulin B, HPV-negative, stage I clinical, and no lymph node metastasis cervical cancer, and the differences were statistically significant ($P<0.05$). However, there were no statistically significant differences in the levels of serum ITF, IBA-1, or Calgranulin B among cervical cancer patients of different ages, pathological types, maximum tumour diameters, depths of myometrium invasion, vascular invasion, or nerve invasion ($P>0.05$; see *Table III*).

Comparison of the serum ITF, IBA-1, and Calgranulin B levels between the death group and the survival group

After a year of follow-up, all patients were divided into two groups based on their ultimate outcomes: 80 patients were in the death group, and 176 were in the survival group. The levels of serum ITF, IBA-1, and Calgranulin B in the death group were significantly greater than those in the survival group ($P<0.05$), as shown in *Table IV*.

Discussion

Cervical cancer ranks second among malignant tumours in women (13). Conventional surgical treatment, radiotherapy, and chemotherapy have limitations (14). The 5-year survival rate is still relatively low. With the popularization of cervical cancer screening and the use of HPV vaccines, cervical cancer incidence and death rates have dramatically declined (15–17). However, cervical cancer patients are getting younger, and the disease's incidence and death rates are continuously rising yearly. Consequently, lowering the death rate from cervical cancer requires early detection and treatment. The levels of tumour markers can significantly increase in malignant tumours (18). Continuous monitoring helps differentiate between benign and malignant tumours. However, traditional tumour markers, such as carbohydrate antigen 125, carcinoembryonic antigen, and cytokeratin 19 fragment, lack specificity and sensitivity in the diagnosis of cervical cancer (19). Therefore, the search for tumour markers with strong specificity and high sensitivity has become a hot topic among scholars.

ITF is a member of the clover family and a novel intestinal peptide secreted by goblet cells (20). It is located on the surface of the intestinal mucosa and has anti-acid, anti-heat, and anti-protease digestion functions. When gastrointestinal ulcers occur, ITF levels increase, and ITF is a protective factor for the gastrointestinal mucosa. ITF is also highly expressed in malignant tumours and functions similarly to oncogenes (21–23). The main manifestations are that ITF promotes tumour initiation and progression, inhibits tumour cell adhesion, promotes tumour cell infiltration and invasion, and significantly inhibits apoptosis, thereby promoting the metastasis and spread of cancer cells (24–26). Moreover, when the cut-off value of serum ITF was 63.60 μg/L, the sensitivity for diagnosing cervical cancer was 71.1%, the specificity was 89.3%, and the AUC was 0.867, indicating that ITF has relatively high auxiliary diagnostic efficacy in diagnosing cervical cancer, which is consistent with the finding that the serum ITF level has specific value in the diagnosis of non-small cell lung cancer. Studies (27–29) have shown that ITF levels in cervical cancer cells are significantly elevated, and that upregulating ITF expression can promote cell proliferation and migration and inhibit apoptosis. The serum ITF levels of poorly differentiated, HPV-positive, stage II clinical and lymph node metastasis cervical cancer patients are significantly greater than those of well-differentiated, HPV-negative, stage I clinical and no lymph node metastasis cervical cancer patients (30). According to one study, the more ITF is expressed in the tissues of stomach cancer, the worse the prognosis of patients, indicating that ITF is a prognostic indicator for various tumours.

IBA-1 is a novel inflammatory mediator first discovered in grafts, derived mainly from mononuclear macrophages, and involved in the body's immune response (31). Studies (32–34) have confirmed that IBA-1 is closely associated with tumours, participates in the regulation of immune responses, promotes cell migration, enhances cell proliferation, regulates the cell cycle, promotes angiogenesis, and is involved in the formation of the tumour microenvironment. The results of this study revealed that the serum IBA-1 level in the cervical cancer group was significantly higher than that in the CIN and healthy control groups (35). Moreover, when the cut-off value for IBA-1 was 251.98 pg/mL, the sensitivity for diagnosing cervical cancer was 77.6%, the specificity was 84.3%, and the AUC was 0.878, indicating that the serum IBA-1 level has relatively high auxiliary diagnostic efficacy for cervical cancer. IBA-1 is highly expressed in breast ductal carcinoma and promotes tumour cell proliferation and migration. These findings indicate that the serum IBA-1 concentration is a prognostic indicator for cervical cancer. Therefore, IBA-1 may be a potential target for regulating tumour metastasis.

Calgranulin B is expressed mainly in vascular endothelial and smooth muscle cells. Moreover, when the cut-off value for serum Calgranulin B was 159.04 ng/mL, the sensitivity for diagnosing cervical cancer was 89.4%, the specificity was 65.6%, and the AUC was 0.847, indicating that serum Calgranulin B has high auxiliary diagnostic efficacy for cervical cancer. The results of this study revealed that the serum Calgranulin B levels of poorly differentiated, HPV-positive, stage II clinical and lymph node metastasis cervical cancer patients are significantly greater than those of well-differentiated, HPV-negative, stage I clinical and no lymph node metastasis cervical cancer patients. These findings indicate that serum Calgranulin B is a prognostic indicator for cervical cancer. The combined detection of ITF, IBA-1, and Calgranulin B has greater diagnostic efficacy for cervical cancer than individual indicators. This indicates some complementarity among the three indicators, and the exact nature of the connection requires further research. This study also has shortcomings: the number of cases was relatively small. Additionally, the markers above should be identified at various follow-up phases in subsequent studies to track dynamic shifts in ITF, IBA-1, and Calgranulin B levels in cervical cancer patients.

Conclusion

Serum ITF, IBA-1, and Calgranulin B levels are highly clinically valuable for cervical cancer diagnosis and prognosis assessment. Enhancing the auxiliary diagnostic efficacy of cervical cancer can be achieved through combined detection.

Authors' contribution

Yuxiang Zhou and Jianwei Liu contributed equally as first co-authors.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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