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A Study on Protein Expression in Cervical Cancer by Using Proteomic Analyzers

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Abstract

Background: In cervical cancer, more than any other type of cancer, the effects of prevention, early diagnosis and prompt treatment is apparent on reduction of mortality.
Objectives: This study aims to achieve protein biomarkers which can be useful in diagnosis and treatment of cervical cancer.
Methods: In this Descriptive study, out of 3 patients who had a positive HPV PCR test, with symptoms of cervical tumor, samples of healthy and cancerous tissues were obtained to perform proteomics. By standard separation techniques, total tissue proteins were purified and separated by using two-dimensional electrophoresis.
Results: Proteomes of healthy and cancerous tissues were compared with each other and the level of expression of considered proteins was evaluated using the required analyses. A total of 173 proteins in gels of two-dimensional electrophoresis was determined, 37 of which had a significant difference in expression. Because descriptive essay. We compared and statistical tests do not make sense.
Conclusions: It seems that in this disease, the expression of a large number of tissue proteins in cancerous tissue is changed, and most of them reduced or not expressed at all. These changed proteins can be useful as a marker in identification and treatment of this disease.

Keywords: Protein Expression, Cervical Cancer, Proteomics Analyzers

1. Background

Cervical cancer is the second common cancer of the female genital organ and the fourth cancer after lung, breast, colon and uterine cancers worldwide which constitute about 6% of all cancers in women.

Global distribution of cervical cancer is high in a way that annually about 490.000 new patients are identified and reported by CDC and WHO [1]. Cervical cancer is the second common cancer among women and constitutes almost 12% of cancers common in women [2]. However, in Iran, there is no detailed and accurate statistics on number of cancerous individuals (women population), mortality rate due to cancer and the relationship between this disease and Human papillomavirus [3].

In cervical cancer, more than any other type of cancer, the effects of prevention, early diagnosis and prompt treatment is apparent on reduction of mortality [4]. Since 1970, human papilloma virus is introduced as the most important environmental cause of this cancer [5]. Ways of transmission of this virus includes sexual transmission and 15 high-risk types of HPV are responsible for 99% of cervical cancers [6]. Yet, other risk factors such as having multiple sexual partners, immunodeficiency, oral contraceptive use and smoking are among considered factors causing cervical cancer [7, 8].

Cervical cancer is a disease in which malignant tissue growth arises from the cervical area, proliferates irregularly and upward and leads to its loss. Since this cancer is one of the diseases without noticeable and obvious symptoms, it may be diagnosed very late [9].

Carcinogenesis mechanism of cervical cancer occurs by DNA of virus which usually does not attach to DNA of cell in non-cancerous cells or precancerous lesions, but viral attached DNA copies are present in viral cells. Early proteins of virus including E6 and E7 are synthesized in cancerous tissue. These proteins along with cellular Rb and P53 proteins create complex [9].

Many studies have shown that two-dimensional electrophoresis can determine the differences between proteome of normal and cancerous cells, the extent of these changes are to be rewarding [10]. In present study, for the first time, by using proteomic techniques, protein factors of cervical cancer were studied and analyzed and the extent of the gene expression difference between normal cells of the cervix were scrutinized. The aim of this paper is to report only protein expression in Cervix cancer

2. Methods

In this Descriptive study, sampling was done during surgery of 3 women who had a positive HPV PCR test with genital lesions and suspected cervical cancer who had referred to Al-Zahra Hospital, Rasht during first six months of 2011, at the same time, sampling of healthy part of the tissue was done which was used as control after confirmation by pathology laboratory. In order to eliminate confounding parameters, healthy and cancerous tissue was obtained from patients in each sampling. Cervical cancer was confirmed by pathologist and Gynecologist.

For protein extraction, frozen healthy and cancerous tissues of patients under liquid nitrogen condition were powdered completely. The resulting powder with lubricating buffer containing Tris-Hcl, magnesium chloride, EDTA and phenyl methyl sulfonyl fluoride (PMSF) and 5 mM beta-mercaptoethanol, 0.5% CHAPS, and 10% glycerol was kept in ice for 30 minutes. Then, the solution was centrifuged in 16,000 G round at 4°C for 30 minutes and protein assay was performed by Bradford technique [11].

For a two-dimensional electrophoresis, the sample was washed three times with PBS buffer and placed in 300 microliters of lubricating buffer (7 M urea, 2 M Thurea urea, 4% (CHAPS), 0.2 -0.3 DTT, 1% - 2% Ampholine with 3-10 Ph) and was shaken for an hour in room temperature. Then, the lysed solution was centrifuged by 10,000 Ground. The solution was maintained in 20°C. In order to test the accuracy, for each healthy and ill case, it was repeated 3 times. Dry Strip was placed in the buffer for a single night to be dewatered. Sample was also taken during the dewatering. Then, based on (Bio-rad) IEF-IPG system, two-dimensional electrophoresis was performed. Next, gels were placed in balancing buffer for second level of electrophoresis. Strip was placed on the second level gel with and was fixed with 0.5% Agarose in electrophoresis buffer containing SDS (25 mM Tris, 192 mM glycine, 0.1% SDS), then started to work vertically.

For protein staining by Coomassie Blue stain, following electrophoresis gel was placed for 6 hours in a solution of 40% methanol, 10% acetic acid and 0.025% Coomassie color which is filtered by using Whatman paper filter. Gel was placed in bleaching solution continuously until the discoloring of the entire field.

Analysis of image spots (proteins) which appeared on the gel was done based on following steps: scanning the gel image, identifying protein spots and quantifying (evaluating the color intensity of spot), matching gels, data analysis, data interpretation and finally creation of two-dimensional electrophoresis databases [12].

Statistical analysis: Because descriptive essay. We compared and statistical tests does not make sense.

Ethical considerations: Objectives of the study were explained in plain language for patients and written consent was obtained from them. Patient information is kept confidential and test if she was willing to inform. Examples of scientific information relevant to the study variables, without restriction of any kind, patient characteristics, and only by a proprietary code was used and was archived as normal.

Clinical trial registration: This thesis is executed by Azad Islamic University of Tonekabon. The authors hereby express their special thanks to Research department of this university for approving this project on October 7, 2010. This study is extracted from an MSc thesis of the same university.

3. Results

Cervical tissue after extraction and its proteome were studied by 2-DE technique. The results of this study are based on the comparison of two gels resulting from two healthy and cancerous tissues of 3 patients. The aim of this paper is to report only protein expression in Cervix cancer.

Two-dimensional gel electrophoresis of normal tissue (Figure 1) and tumor tissue of human cervix are depicted after identifying the spots (Figure 2). In the Figure 3 shows the two-dimensional gel electrophoresis after numbering which indicate the proteins obtained by gel analysis and matching them with the databases. As shown in the image, Expression of 8 (21.6%) proteins in cancerous group is suppressed. Also, 6 (16. 2%) proteins are expressed in cancerous sample which had no expression in healthy one. 12 (32.4%) proteins in cancerous sample indicate increase in expression while 11 (29.7%) others are indicative of decrease in expression (Table 1).

Also based on Figure 3, expression of 8 proteins (1,2,9,24,69,131,136,168) is suppressed in cancerous group. 6 proteins (25,61,62,77,78,112) are expressed in a cancerous case with no expression in healthy one. 12 proteins (144,145,146,173,5,31,32,107,113,128,135,143,) showed increase in expression in cancerous sample and 11 proteins (10,11,14,19,38,42,44,71,106,167) decrease in expression (Table 2).

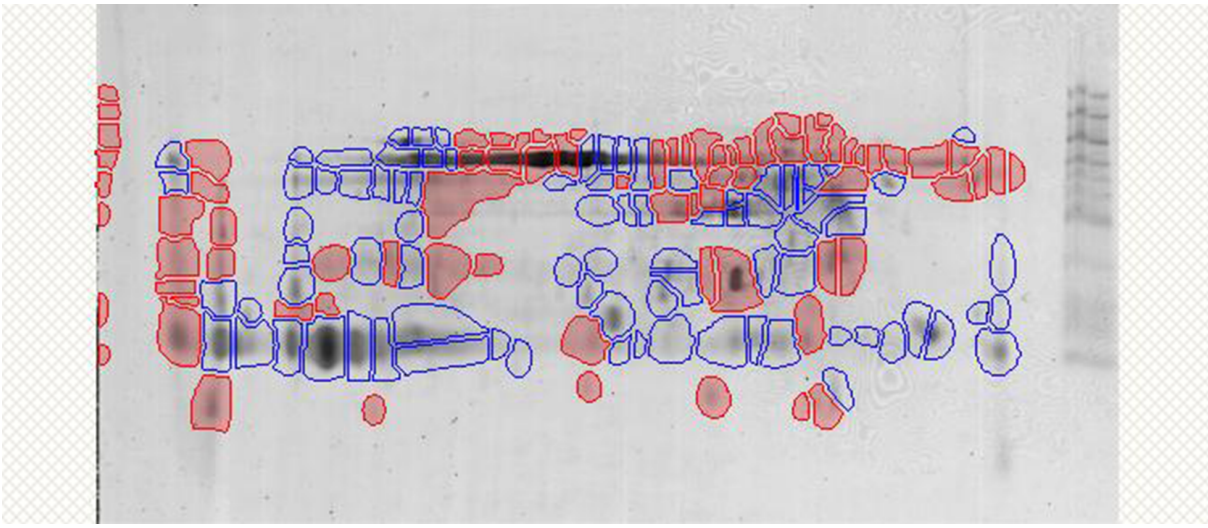

Figure 1. Two-Dimensional Gel Electrophoresis of Normal Tissue

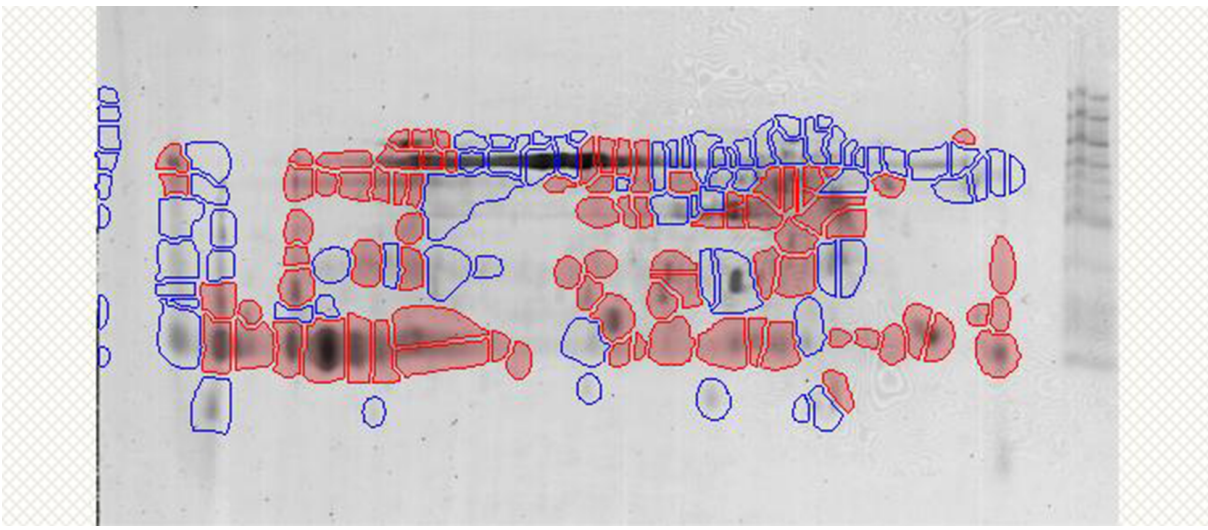

Figure 2. Tumor Tissue of Human Cervix are Depicted after Identifying the Spots

Table 1. Classification of Proteins Found

	No. (%)
Proteins that expression is suppressed	8 (21.6)
Proteins that are expressed	6 (2.16)
Proteins that expression is increased	12 (4.32)
Proteins that expression is decreased	11 (7.29)
Total	37 (100)

Spots analysis by Flicker software and based on fold more than 2 P value less than 0.05.

4. Discussion

The goal of proteomics is to study the whole protein content of a biological sample in a series of experiments. This technique can be a valuable approach to understanding the complex responses of an organism to a stimulus. In recent years, two-dimensional electrophoresis methods,

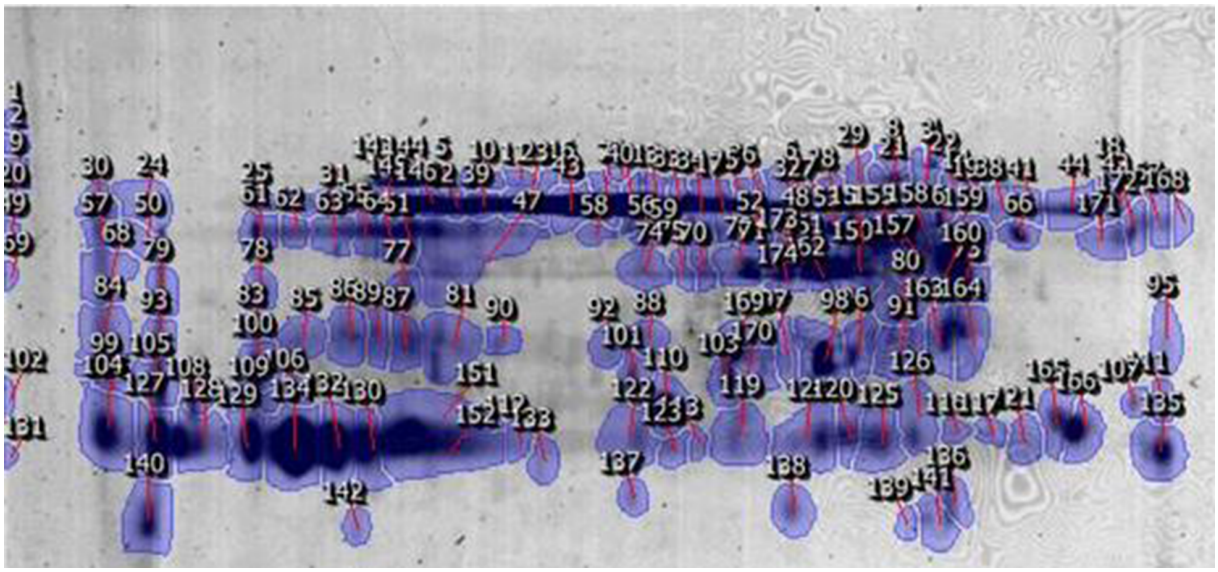

Figure 3. Numbered Spots on Electrophoresis

Table 2. Marking Proteins Expressed by Type

	Num	Spot Numer
Proteins that expression is suppressed	8	1,2,9,24,69,131,136,168
Proteins that are expressed	6	25,61,62,77,78,112
Proteins that expression is increased	12	5,31,32,107,113,128,135,143,144,145,146,173
Proteins that expression is decreased	11	10,11,14,19,38,42,44,71,106,167

Copper spectrophotometry, and bioinformatics are widely used for research on proteins.

Despite of accessibility to these systems, Processes of this technology have limited ability to isolate protein complexes and proteins in low amount but with developments going on we can cope with such problems [13]. Many studies have shown that two-dimensional electrophoresis can determine the differences between proteome of normal and cancerous cells, the extent of these changes seem valuable [10]. Proteomics-based diagnostic advances have revolutionized the field of molecular medicine, which not only can be used for diagnosis, but also for clinical aspects [14].

Many researchers using proteomics technology in study on all cancer types, comparison and identification of polypeptides in normal and cancerous tissue, have found biochemical markers suitable for diagnosis of degree, type and amount of damage. Regarding the fact that no proteomic studies have been conducted in the area of cervical cancer now by having effective methods such as two-dimensional electrophoresis, measures can be taken to identify the markers of this type of cancer so that the best and most appropriate treatments be done before worsening of the disease such as in lung cancer cases where treatment can be initiated by identifying markers and diagnosis by using two-dimensional electrophoresis [15, 16].

Many studies have shown that some types of high-risk HPV types are known to play an important role in the development of cervical cancer, in a way that these types are known in more than 99% of cervical cancers worldwide [17]. But, unfortunately, no comprehensive statistics about the prevalence of oncogene types of this virus associated with cervical cancer are existed in Iran [18].

In a study by Mohit raja and his colleagues in 2008 on the quality of proteomic analysis of fixed tissues by formalin and paraffin in patients with HIV with oral HPV manifestation, it was reported that samples fixed in paraffin compared to samples kept in nitrogen 96° and then protein extraction and steps of proteomic are performed on them have a lower quality. Therefore, they recommended proteins extraction from tissue using freezing by nitrogen method [19]. Thus, in this study, the frozen samples in nitrogen 96 were used. Separated proteins of healthy and cancerous tissue were analyzed by two-dimensional electrophoresis. Gels shown in figures mentioned have the ability to be compared and analyzed bioinformatically due to which points on two gels of normal and cancerous tissue were compared by Flicker software. With the help of this software, 173 points in normal tissue and tumor tis-

sue of the cervix were detected, of which 37 points show a significant difference. Expression of 8 proteins in cancerous group is suppressed. Also, 6 proteins are expressed in cancerous sample which had no expression in healthy one.12 proteins in cancerous sample indicate increase in expression while 11 others are indicative of decrease in expression. Such as Idanya Serafin-Higuera and et al 's study which at first by the fluorescent two-dimensional electrophoresis (2D-DIGE) they found an area with a differential expression pattern among samples was located, from which 129 spots with a diminishing expression and 150 with increased expression, 53 spots showed a greater differential expression and the identification of the ten spots was achieved by Mass Spectrometry (MALDI-TOF) and at least they found proteins such as While Keratin, type II cytoskeletal 5, Peroxiredoxin-1 and 14-3-3 protein sigma showed a decrease in their protein expression level in cervical cancer in comparison with normal cervix cells [20].

In another study, Jae Yun Song was studied on three cervical cancer tissue samples and three normal cervical tissue samples were obtained and protein expression was compared and was identified in the samples with the use of matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). A total of 20 proteins that showed up-regulated expression in the cervical cancer tissue samples were selected and identified. Seven proteins were matched to allograft inflammatory factor 1 (AIF-1), actine-like protein 2 (ALP2), brain type fatty acid-binding protein (B-FABP), NCK adaptor protein 1 (NCK-1), islet cell autoantigen 1 (ICA69), cationic trypsinogen (PRSS1), and cyclin-dependent kinase 4 (CDK4), but the remaining 13 proteins were unidentifiable [21].

At 2014 Xia Guo and et al. comparative proteomics based on two dimensional differential in-gel electrophoresis (2D-DIGE) was employed to quantitatively analyze plasma proteins of healthy women and with early stage cervical carcinoma. The 2D-DIGE image were analyzed statistically using DeCyder™2D software. The statistical analysis of proteomic data revealed that 43 protein spots showed significantly different expression (ratio > 1.5, $P < 0.01$). A further identification of these protein spots by MALDI-TOF-MS found out 16 different proteins. Bioinformatics analysis showed that 10 plasma proteins as candidate biomarker were screened, mainly including lipid metabolism-related proteins (APOA4,APOA1, APOE), complement (EPPK1, CFHR1), metabolic enzymes (CP, F2,MASP2), glycoprotein (CLU), and immune function-related proteins (IGK@) [22].

On the other hand Van Raemdonck in Belgium was identificated protein biomarkers for cervical cancer by using human cervicovaginal fluid. He used a differential proteomics study was set up using CVF samples from healthy and precancerous women and found the proteome analysis revealed 16 candidate biomarkers of which alpha-actinin-4 and pyruvate kinase isozyme M1/M2) were most promising [23].

Therefore, 37 diverse points from comparison of tumor and normal tissues of cervix demonstrates a major change which occurs in body following a tumor tissue. In other words, differences in expression of the proteins during incidence of the tumor reveal that the regulation of large number of genes is altered. Difference between gene expressions in two gels can be useful in achieving the protein markers for the detection and treatment of patients with this type of tumor. Simultaneous change of many proteins in this disease can indicate biochemical and mechanisms involved in incidence of this disease which can be effective for medicinal purposes in the treatment process [24]. Furthermore, by using proteomic methods, creating biological kits will help the diagnosis and recognition of the disease.

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Footnotes

Conflict of Interest: All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest: The authors declare no conflict of interest.

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