A Comparison of Protein Profiles of Cervical Tissue Homogenate, Exfoliated Cells from Cervix and Serum in Normal and Cervical Malignancy Conditions

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Cervical cancer, the second most common cancer in women, progresses silently over long periods before producing any clinical manifestation. Research in early detection of this condition using proteomic techniques is of very recent origin. We used high-performance liquid chromatography combined with laser-induced fluorescence method in our lab to record the protein profiles of tissue homogenate, cell lysate and serum samples of normal and different stages of malignant conditions of the cervix. Information on protein markers in the protein profiles was derived using various data processing methods including curve resolution. The variations in relative intensities of different peaks with respect to peak height, width and area under the curve from different sample types were compared to get information regarding the concentration of the various proteins and their significance in the induction and metastasis of cervical cancer. The method can be used in diagnosis, follow-up with respect to the progression, remission and effective therapy, in cervical malignancy.

Introduction

Cervical cancer derives "from the multi-layered cervical epithelium which has an organization similar to that of the epidermis of the skin" (1). Squamous cell carcinoma is the most common type of cervical malignancy, accounting for 85–90% of all cases. This usually originates at the squamo-columnar junction where the part of the cervix that connects with the vagina (ectocervix) meets the part that opens into the uterus (endocervix). At this location, the epithelium is of the stratified squamous type, with an outermost layer of squamous cells, followed by an intermediate layer of polyhedral cells, after which comes the basal layer, and then the basal lamina, below which we have the well-vascularized lamina propria (connective tissue) (2). Under normal conditions, proliferation occurs only in the basal layer, the cells then moving outward to the surface. However, "it is not unusual to find patches of dysplasia where dividing cells are no longer confined to the basal layer" (1) and cells in abnormal, early stages of differentiation are sloughed off from the surface. When dysplasia progresses to carcinoma in situ (CIS), we have proliferating cells in all the layers of the epithelium, and the sloughed off cells contain large number of undifferentiated, proliferating cells. When the disease turns into truly malignant cervical carcinoma, the abnormal cells, in addition to sloughing off from the surface, break out of the basal lamina and invade the connective tissue, metastasizing to other regions of the body. It can thus be seen that both biopsy tissue and exfoliated cellular samples will give progressively increasing changes from normal, through dysplastic, CIS and malignant conditions.

Cytological smears used for diagnostic applications come from cells exfoliated from the surface of the epithelial tissue, while cells which enter the blood stream are thought to do so from the malignant cells near the basal lamina, that too, only those, equipped with required enzymes which will enable them to penetrate the collagen barrier. One may assume that, since the abnormally proliferating cells enter the main bodily system, penetrating the basal membrane, in malignant condition only, drastic changes will not be seen in circulating body fluids such as blood before this stage. However, there are a few factors which show this assumption to be not very correct. It is well known that (3) even in very early stages of malignancy, some of the abnormal cells enter the circulation and migrate to other parts. As mentioned earlier, even under dysplastic conditions, some of the dysplastic cells migrate to the outermost layer and get sloughed off. It is quite likely that these are exposed to various internal organs some of these will enter the circulatory system. The molecular mechanisms of carcinogenesis involve exaggerated expression of several proteins such as various growth factors, growth factor receptors, protein kinases and G proteins. This, combined with the loss of so-called tumor suppressor genes, leads to considerable changes in the protein profile of body fluids such as blood. Both herpes simplex virus Type II and human papilloma virus Types 16 and 18 are implicated in cervical malignancy. Viral antigens and corresponding antibodies are also thus expected to be seen in blood in cervical malignancy. It has been known for a long time now that living beings pass a life code, in the form of DNA, from one generation to the next. It is also being realized now that sets of instructions—written in proteins and enzymes—that tell each cell of the body which genes should be expressed, also are passed on from one generation to the next (4). Serum, cell lysate and biopsy tissue homogenates should thus show protein profiles varying over a wide range of molecular species at different levels of concentrations as the disease progresses from dysplasia through cervical intraepithelial neoplasia, CIS and invasive carcinoma. It will thus be informative to compare the protein profiles of serum, tissue and cytological smear under same conditions of normal/malignant states.

Several groups are working at present in this area of detection of cervical cancer using different techniques such as two-
dimensional gel electrophoresis, MALDI-TOF mass spectrometry, high-performance liquid chromatography (HPLC)–MS and SELDI-MS (5–8). In our laboratory, we have been able to develop HPLC combined with laser-induced fluorescence (HPLC-LIF) method for recording chromatograms (protein profiles) of clinical samples. The recorded samples were analyzed using principal component analysis (PCA). Three parameters such as scores of the factors, squared residuals and Mahalanobis distance derived from PCA were used to discriminate the normal and malignant samples from cervical (9–12), breast (13), ovarian (14) and oral (15, 16) cancers. In our previous studies, we have showed that the Match/No Match test of samples with normal/malignant standard set of chromatograms can be used for the objective discrimination of normal, malignant and other disease conditions of the cervix (9–12). The HPLC-LIF technique can look at the proteins present in the sample in a single run with very high sensitivity, without any of the disadvantages associated with morphological studies. The present system can detect the proteins even if they are present in femtomole quantities (15). The method is quite fast, the time required for analysis of one sample being less than an hour. The analysis can be done by a trained technician and can be automated for continuous operation. The diagnosis is done by mathematical analysis of the complete protein profile (not just one or two proteins) and is thus highly objective since it does not depend on personal observation or experience. In view of the several advantages of our ultra-sensitive HPLC-LIF method over the other methods (17), we have employed this technique for protein profiling of serum, tissue homogenate and cell lysate for early detection of cervical cancer. Our studies have shown that protein profiling of serum, cytological specimens and biopsy tissue can discriminate between normal, malignant and other disease conditions with high sensitivity and specificity. In the present paper, we have compared the protein profiles in these three types of samples (serum, tissue homogenate and cell lysate) in normal and different stages of malignant conditions, and also in diseases of the cervix other than malignancy. The results are presented and discussed below, from the point of view of possible correlations between these three classes of samples.

**Experimental**

The details of sample collection, processing and use have been described earlier (9–11). Normal tissue samples from the squamo-columnar junction of cervix were obtained from subjects who underwent hysterectomy, for reasons other than malignancy. Biopsy tissues from malignant subjects were collected from patients visiting the hospital. All the samples, irrespective of whether they belonged to normal or malignant subjects, were transported to the lab, immediately after collection, in normal saline. In the lab, the tissues were washed with normal saline (0.9% sodium chloride) several times to remove any traces of blood. The samples were weighed and minced with 20% wet weight of Tris–EDTA buffer (0.1 M, pH 7.4). They were then homogenized by a manual homogenizer (18 blade IKA-WERKE) and centrifuged at 5,000 rpm for 20 min twice. Supernatant was collected through a syringe fitted with 0.45 μm filter. Normal and malignant/abnormal cervical condition cellular samples (exfoliated cells of the cervix) were collected from healthy volunteers and patients who visited the hospital. Cellular samples were collected from the outside and just inside the opening of the cervix (cervical canal) by gently scraping with a wooden spatula.

Normal samples of serum were collected from volunteers who were judged to be clinically normal with respect to cervical cancer, and age matched, as far as possible. All normal subjects did not have any diagnosed disease. Malignant samples were collected from subjects diagnosed with cervical cancer. Blood samples were transported to the laboratory immediately after collection. The samples were stored at room temperature in an upright position for ~30 min. The separated liquid portion was centrifuged at 3,000 rpm for 5 min. The samples were diluted 100/500 times with HPLC-grade water. Twenty microliters of the diluted sample were injected into the HPLC system.

If storage was necessary, samples were stored at -80°C in the deep freeze. They were passively thawed to room temperature just before use. We have verified that the chromatograms (protein profiles) of samples did not show any change, even when recorded after several weeks of storage in the deep freeze. All samples were used with informed consent. Clearance of the Ethical Council of the Institute was obtained for these studies. All samples were obtained from the Department of Obstetrics and Gynecology, Kasturba Hospital, Manipal, India. Serum, cell lysate and biopsy tissues were collected from age matched normal and patients suffering from various stages of cervical cancer. Table I gives the details of the samples. A total of 25 normal and 33 malignant serum samples, 15 normal and 29 malignant biopsy tissue samples and 13 normal and 15 malignant cell lysate were collected from normal and cervical cancer patients. The cell lysates were also collected from eight patients suffering from various conditions of the cervix other than malignancy such as inflammation, erosion and nabothian cyst to compare the protein profiles of these groups from normal and malignant conditions of the cervix. These were categorized into a totally different group, namely “others”.

**Instrumentation and reagents**

Details of our ultra-sensitive HPLC-LIF system are given elsewhere (10, 11). The experimental set up consists of an HP1100 HPLC system with Rheodyne 7725 manual injector (20 μL loop) and 219TP52 Biphenyl narrow bore column (bonded phase: diphenyl, column dimension: 2.1 \times 250 \text{ mm}, \text{ particle size: } 5 \text{ μm, pore size: } 300 \text{ Å}). The effluent from the column is passed through a quartz capillary flow cell designed and fabricated in our lab. Excitation of proteins is done with 257 nm radiation (10 mW) from a frequency-doubled Ar+ laser (Coherent Innova 90C Fred). Fluorescence is collected from the two sides orthogonal to the laser beam. The protein profile is obtained by recording

| Table I |
| Sample Details |
| Sample type | Normal | Stage I | Stage II | Stage III | Stage IV | Others* |
| Serum | 25 (38 ± 12)* | 2 | 13 | 17 | 1 | NIL |
| Cell lysate | 13 (38 ± 10.6) | NIL | 4 | 11 | NIL | 8 |
| Tissue homogenate | 15 (38 ± 11) | 2 | 7 | 19 | 1 | NIL |

NIL, no samples.

*Others", the number of subjects with cervical diseases other than malignancy.

**Average age and mean deviation.
fluorescence at 340–350 nm as a function of time, using a JY DH10 double monochromator, Hamamatsu R453 Photomultiplier, EG & G 651 chopper (20 Hz), EG & G 5113 preamplifier and EG & G 7265 lock-in amplifier. All HPLC-LIF measurements were done with following conditions: laser power at flow cell 5 mW; monochromator set to 350 nm, band pass 14 nm; photo multiplier tube (PMT) voltage 850 V. The sample was eluted under a gradient run with A: (water, 0.1% v/v, TFA) 70–40%; B: (acetonitrile, 0.1%, v/v, TFA) 30–60% in 0–60 min, followed by B 60–100% in 1 min. The 100% acetonitrile run was continued for another 14 min taking the total run time to 75 min. After each run, column was regenerated with HPLC-grade water with 0.1% TFA, for 15 min. The flow rate was 200 μL/min. The same gradient runs were maintained for all three types of samples.

Simultaneously with the chromatographic profile, fluorescence from the opposite side is used to record the fluorescence spectrum of each protein, using ACTON Research Corporation SpectraPro-2150i, 0.150m imaging spectrograph and Princeton CCD (Roper Scientific, Model-RTE/CCD 128-HB) detector.

Methods

For correlation of different classes of samples, run over a period of several months, it is very essential that all data are appropriately brought together in a comparable format. The sample collection and processing protocols and HPLC run procedures discussed earlier were strictly adhered throughout these studies. In addition, we have evolved data processing procedures that ensured minimum errors due to small random variations in experimental procedures over long periods. The data processing involved background subtraction, accurate calibration of chromatograms over the time axis and normalization of all chromatograms. The data processing involved background subtraction, accurate calibration of chromatograms over the time axis and normalization of all chromatograms of a given class with respect to a component which showed least variations from run to run.

The background fluorescence changes during the run because of changes in the relative concentrations of H2O–TFA and acetonitrile–TFA as the run proceeds. This fluorescence most probably comes from biomolecules other than proteins present in the samples and also from the acetonitrile–TFA complex. To facilitate intercomparison of different classes of compounds (serum, cells and tissue), it is preferable to reduce this background contribution as much as possible. This was done by fitting a multipoint polynomial to the slowly varying background and subtracting the resultant background fluorescence curve from all points across the chromatogram.

Due to possible day-to-day variations in the several steps (sample collection, processing for HPLC run, instrumental and chromatographic system conditions, etc.), it is possible that even the same sample may show slight variations from run to run in retention times and peak intensities. To reduce the errors in retention time variations, all chromatograms of a given class were calibrated to the same timescale using suitable peaks, common in all runs, selected across the total time interval. As a result of this calibration, the variations in retention times were reduced to negligible values. For example, the strongest peak—albumin—in serum was shown to have a retention time of 1,527 ± 0.4 s in all of the normal and malignant (different stages) serum samples.

Though clinical samples may contain hundreds of proteins, under normal conditions, each protein will be present within specific concentration ranges only, in all samples of a given class, say, blood. Many of these maintain their relative concentrations in this relatively narrow range. Disease often alters the amount of these proteins in the clinical samples (18). To get a quantitative estimate of those which change due to disease, and those which appear new, all chromatograms of a given class were normalized with reference to a peak which showed the minimum variation from sample to sample. This gives a quantitative estimate of the changes which happen in the various stages of the disease in the different classes of samples.

As mentioned above, though physiological samples may contain the regular proteins in specific concentration ranges only, there can be many other biomolecular species, including proteins, which change from subject to subject randomly, because of differences in lifestyle, temporary aberrations in physiological conditions, unidentified bacterial/viral infections, unanticipated changes in daily routine, etc. Since the present studies are intended to correlate systematic changes due to induction and progression of malignancy in different samples, it is necessary to reduce the variations from such random changes to a minimum and ensure consistent correlations between the three classes of samples. To achieve this, the mean profiles of samples of each class are formed using the base line corrected, calibrated, normalized profiles for all the samples of that class. This will have the effect of improving the signal-to-noise ratio for consistent peaks and reducing random variations, by averaging them over all samples. All data analyses were done with PLS Plus IQ46 (GRAMS/AI Spectroscopy Software, 7.02, Thermo Galactic Corporation, Woburn, MA, USA, 2005).

We carried out curve resolution studies to resolve overlapping peaks from all the three types of samples (serum, tissue homogenate and cellular samples). Mean of normal sample and mean of different stages of malignant samples were taken for curve fitting. Curve fitting is a powerful technique that models or fits a number of ideal peaks to an actual complex contour of overlapping peaks. Physiological samples consist of complex mixture of many proteins and in the chromatogram some of them may overlap to give unsymmetrical contours. Curve fitting techniques can resolve these peaks to a set of individual peaks, which will help in interpretation and data analysis of the protein profiles. We have used the Gaussian function for curve fitting since this gave very good fits with least residuals. This is given by

\[ f(x) = H \exp - \frac{(x-x_0)^2}{w} = \frac{4\ln(2)}{w^2} \]

the above function gives the intensity \( f(x) \), at any point \( x \) as a function of the peak height \( H \), peak position \( x_0 \) and full width at half height \( w \).

Results

Figure 1 shows a typical chromatogram (unprocessed) and examples of fluorescence spectra recorded for human serum albumin and creatine phosphokinase (CPK) "on the fly" for a run with 20 μL of 500 times diluted serum. Some of the chromatographic protein peaks of serum were identified by SDS–PAGE and co-injection method: 1,345 s—transferrin; 1,485, 152—human serum albumin and 1,849—CPK. From Figure 1, it is clear that the HPLC-LIF technique can be used for the simultaneous recording.
of both chromatograms and fluorescence spectra of proteins corresponding to each of the chromatographic peak of a given sample. The relative concentrations of various proteins in the clinical samples can also be obtained by using the fluorescence spectra.

Figure 2 shows the serum protein profile of normal and different stages of cervical cancer. It is clear from the figure that the protein profiles show noticeable differences when we go from normal to the different stages. In the 450–900 s region, many proteins are highly expressed in Stages I and IV cervical cancer. The region between 1,100 and 1,280 shows a big difference when we go from normal to Stage IV cervical cancers. In malignant samples (Stages I–IV), the doublets from the region 1,202 and 1,243 s is found to be stronger. The region between 500 and 800 s and 1,750 and 2,250 s shows mostly unresolved peaks. The 1,700–2,000 s region shows noticeable variation from normal to different stages of the cancer. The intensity of CPK peaks (at 1,849 s) are found to be greatly increased in all the stages of cervical cancer.

The typical curve fitting for normal tissue homogenate for the region 1,320–1,720 s using Gaussian function is shown in Figure 3. It is clear from the figure that the two unresolved peaks from 1,450 to 1,620 regions were resolved by this method. Curve fitting is also useful in determining the exact peak positions, widths, heights and areas of the individual components in a set of overlapping peaks.

Figure 4 shows the mean protein profiles of all three types of samples from each class—serum, cell lysate and tissue homogenate—from normal subjects. It should be noted that the three types of samples are quite different from each other. We observed that all the 25 serum samples, irrespective of the age, show more or less same profile, especially with respect to the peaks coming from the major proteins such as albumin, globulins and transferrin. The tissue homogenates also resemble each other fairly well, whereas the cell extracts show noticeable differences from one another. The range of variations in protein profiles in samples of each class can be seen better in Figure 5, which shows the sum of squared differences, \( \sum (M_i - P_i)^2 \), where \( M \) is the mean of all profiles of a given class and \( P \) is an individual profile in that class. Considering that the sum is over all points (> 1,000) of the profile for a sample, it is seen that the difference at any given point from the mean is almost zero for serum, slightly larger for tissue homogenates and noticeable only for cell lysates. This is understandable, since it is well known that many accessible parts of the female genital system are vulnerable to nonspecific microbial and dermatological disorders (19), and these external "contaminations" will have more effect on the cellular samples. Also the cervix is often prone to dysplastic conditions because of chronic infection (20).

As explained earlier, to understand the correlations between the three classes, it is thus better to look at the mean profiles in each class. Some of the major serum proteins are indicated to give an idea of the sensitivity of the system. For example, the albumin peak, in 20 \( \mu \)L of 500 times diluted serum gives a signal of > 1 V. The noise level in our system is \( \sim 0.001 \) V, and this gives a limit of detection (in these runs) of a few femtomoles. We have shown (10–17) that the sensitivity of the system can be increased by 2–3 orders of magnitude, if necessary, by appropriate choice of experimental parameters (higher laser power, multi-passing laser, collecting more fluorescence with better optics, etc.) and detection system (higher PMT voltage, more signal amplification, use of band pass filters in place of monochromator, etc.). The very high sensitivity of the system, as it is, can be seen from Figure 6, which gives the same profiles of Figure 4 in a 10 times expanded scale highlighting the peaks of proteins present only in very small amounts, still with practically very little background noise.

Comparison of the protein peaks of serum, cell lysate and tissue homogenates from normal subjects, with few peaks which appear at the same retention times (± 5 s) in two or all three, is shown in Table II. The protein peaks observed in cells/tissue can be used to check whether the blood has picked up any of these proteins. The most interesting observation is that there is not much consistent correlation between the protein peaks of the three classes of samples except for few serum peaks.
The protein peaks observed in these samples can be divided into four classes: proteins present in normal serum and appear without much change in their levels in malignancy conditions; proteins which are present in normal serum, but either increase or decrease in their levels in malignancy; proteins present in normal condition but absent in malignancy and finally, proteins which were absent (or present in extremely small amounts) in normal serum and appear clearly in malignancy. It is clear from the table that some of the protein peaks at 361, 862, 1,201, 1,240, 1,344, 1,485, 1,527, 1,535, 1,665 and the last three peaks from 2,400 to 2,580 s regions are present in all the stages of the serum samples including the normal samples. Some of these are the basic serum proteins such as albumin (1,485, 1,527), transferrin (1,344) and globulins from 2,400 to 2,580 regions. We have identified these peaks by coinjection method by using commercially available proteins. It is interesting to observe that the area under the curve increases for all the peaks when we move from normal to malignant conditions. The peaks at 468, 1,533 and 2,440 s regions are observed only in the later stages of cervical cancer.

Table IV shows the results of cell lysate. As we have mentioned (Figure 4), protein profiles of the cell lysates are more complex compared with the other two sample types. Since we have taken the average protein profile of each class (normal, malignant II and III and other diseases conditions) to study the changes in the protein profile, variation among its own members of the class is minimized. Noticeable differences observed from normal to disease conditions and from that to the malignant condition.

Figure 2. Protein profile of the mean serum samples. (A) Normal, (B) Stage I, (C) Stage II, (D) Stage III and (E) Stage IV samples (expanded scale from 0 to 0.2). Broken lines show changes in the peak intensity of some proteins during malignant conditions.
enable one to distinguish between malignant and other disease conditions. It is interesting to observe many new peaks in malignant Stages II and III conditions, which are totally absent in the normal condition. The 1,866 peak is found to be observed both in normal and malignant conditions. Certain protein peaks (1,265, 2,280) are observed in normal and subjects with various disease conditions and certain other peaks, in malignant and disease conditions (1,257, 1,804).

Table V shows the results for the tissue homogenate. In the normal tissue except for the peak at ~1,600, as shown in Figure 4, other proteins are present only in small amounts. On the other hand, malignant tissue homogenates show large increase in many proteins. It is seen that many protein peaks are expressed in later stages of cervical malignancy (Stages III and IV) peaks at 480, 1,595, 1,728, 2,172, etc.

Discussion
Table II shows the curve resolution results of retention times of common mean protein peaks from serum, cell lysate and tissue homogenate of normal subjects. Since the same elution conditions were used for all three types of samples, peaks having noticeably different retention times are thus most likely to be coming from different proteins. Many normal serum protein peaks are not seen in the normal samples of cell lysate or tissue.
homogenate. To the extent of our sensitivity, thus, there seems to be no protein peaks from serum in the other samples. This indicates that any proteins observed in tissue or cell samples which may correspond to proteins of serum samples are generated in these samples only and not through contamination from blood. Table II also shows the presence of common peaks (1,535 and 1,846 s region) in all the three types of samples. This observation is indicating the probability of escape of these proteins into the bloodstream under normal conditions. Some peaks at 732, 1,527, 1,680, 2,002 and 2,448 s are found in protein profile of serum and tissue homogenate and are totally absent from the protein profile of cell lysate. Likewise, many peaks (420, 565, 1,186, 1,866 and 2,382 s) are having same retention time in tissue homogenate and cell lysate and are absent in serum. This clearly shows that cell lysates and tissue homogenates seem to have different proteins compared with that of the proteins present in the serum. It is thus highly unlikely that there is any contamination from blood in the other two classes of samples. Similar considerations apply for comparison between cell lysate and tissue homogenate. Some of the serum protein peaks at regions 732, 1,681, 2,003, 2,349 and 2,448 s are absent in cell lysate, but they are well expressed in tissue homogenates. This may seem surprising to some extent, but need not be so, since the cell lysate contains shedded off cells only, whereas tissue homogenate contains proteins from cells in the tissue body. Even in malignancy, only very few cells can enter into the bloodstream, since all malignant cells do not have the ability to metastasize to different locations, by passing through the basal lamina. It may be possible to identify them in the malignant serum samples with increased sensitivity, and we are pursuing this aspect at present.

The increase in the area under the curve for majority of the serum proteins, as it is observed from Table III, clearly shows the upregulation of these proteins during malignant conditions to meet the necessary requirements such as growth factors and other proteins required for the fast growing malignant cells. The absence of peaks at 468 and 1,533 s regions in normal and Stage I of cervical cancer and their presence in the last three stages of the cancer shows that they arise due to malignancy.
The 2,440 peak is observed only in Stages III and IV, showing the possibility of their role in metastasis.

The most interesting observation from the protein profile analysis of cell lysate (Table IV) is the presence of protein peak in 1,866 s region, which is expressed in both normal and malignant Stage II cells. This is understandable, because any sample from subjects with disease conditions, including malignancy, will have few normal cells also. The absence of protein peaks in the normal conditions and their presence in the malignant conditions (peaks at 1,894, 1,907 s, etc.) clearly indicates the usefulness of this method to diagnose malignancy.

The protein profile of the tissue homogenate shows many possible markers for the detection of cervical cancer. Many are expressed well in the initial stages of malignancy, indicating their possible role in induction of disease (peak at 807, 2,666 s). The detection of these protein peaks can help in the early diagnosis of the cancer.

Tables III, IV and V were compared with get information on possible proteins which might have passed from the malignant tissues and cell into blood circulation. One of the tissue protein peaks at 971 ± 4 from the first three stages of malignancy was found to be observed in Stages I–III of serum samples. This clearly indicates that this protein can be the possible markers for the diagnosis of cervical cancer. Monitoring the level of this protein in the blood may help in the early detection of metastasis.

In India, eight women die every hour (≏74,000 every year), of cervical cancer, and still only 5–6% undergo any diagnostic check-up. On an average, 120,000 new cases of cervical cancer are detected annually. This calls for an urgent need to develop early
detection methods, by which women in rural areas can be regularly screened in a rural healthcare facilities, which may not have any formal clinical/pathological expertise, and which will be most often managed by a single healthcare technician. Development of a system usable as a screening tool for susceptible population (in this case females), as a diagnostic method for suspect cases, a pathologic tool for biopsy samples and finally, an easy follow-up procedure during and after therapy, is thus highly desirable for improving the survival rate for this cancer. The system has to be fairly rugged, portable, relatively fast, usable by a technician and of sufficient operational expertise, and which will enable the clinician to plan changes in therapy procedures and recurrence of disease. It is thus possible to follow the results of therapy at regular intervals through protein profiling of serum or cytological smears which are readily available any time. This will enable the clinician to plan changes in therapy procedures as and when required.

**Conclusion**

In conclusion, protein profile studies of serum, tissue and cell lysate carried out by HPLC-LIF technique can give information on the changes occurring in the various stages of cervical malignancy. The exact molecular mechanism occurring at cellular level can be well studied by comparing the protein profiles of different sample types. Since the relative intensity, area under the curve and width of the individual peaks change drastically from normal to malignant and other disease conditions. The method can also be used to monitor the efficacy of therapy for various diseases of female genital region including malignant conditions and recurrence of disease. It is thus possible to follow the results of therapy at regular intervals through protein profiling of serum or cytological smears which are readily available any time. This will enable the clinician to plan changes in therapy procedures as and when required.

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