**Research article**

**Immunohistochemical study of CD1a positive dendritic cells in normal, benign and malignant lesions of the human uterus and cervix**

Kalpana Ramachandran¹,²

¹Bharath Institute of Higher Education & Research (BIHER), Chennai, Tamil Nadu, India  
²Department of Anatomy, SRIHER, Porur, Chennai, Tamil Nadu, India

(Received: January 2022  Revised: February 2022  Accepted: February 2022)

Corresponding author: Kalpana Ramachandran. Email: kalpanasriram1@gmail.com

**ABSTRACT**

**Introduction and Aim:** Dendritic cells (DCs) perform the function of antigen-presenting cells of the immune system of mammals. After demonstration of DCs in the human cervix, more focus fell on the role of DCs distributed in various benign and cancerous lesions of endometrium. In our study, we have studied the role of CD1a positive DCs in non-malignant and cancerous tissues of the uterus and human cervix.

**Materials and Methods:** A prospective analysis was conducted on patients who had undergone hysterectomy and cervical biopsy. After a detailed menstrual cycle history, the processed specimens were subjected for hematoxylin-eosin and immunohistochemical staining. The CD1a positive cells were counted per 20 high power fields and the mean cells per high power field (HPF) was calculated.

**Results:** Eighty specimens were studied. Forty-six uterine and 34 cervix specimens were studied. The average chronological age of our study patients was 45.7 years. Bleeding per vagina was the most common presentation. Fibroid uterus was the most common indication for hysterectomy. The minimum and maximum DCs amongst the 80 specimens were 1 and 246 per 20 HPF and the overall mean was 72.7. The mean cervical and uterine DCs per HPF were 7.7 and 0.7 respectively (P=0.0116). The average DCs per HPF in benign group were more than that in malignant specimens (9.23 and 1.76, respectively).

**Conclusion:** A higher concentration of DCs in the cervical tissue was observed. Malignant tissues showed a lesser number of DCs than the benign specimens.

**Keywords:** Cervix; uterus; CD1a; hysterectomy; dendritic-cell.

**INTRODUCTION**

Dendritic cells (DCs) are accessory cells that perform the role of antigen-presenting cells of the immune system of mammals(1). Their main function includes processing the antigenic substance and presenting it on the cell surface of the T-helper cells of the mammalian immune system. They serve as intermediaries or messengers between the adaptive and the innate immune systems of our body (2). It is a special type of phagocyte and a type of antigen presenting cell. These cells originate from their progenitors of bone marrow from where they drift to the lymph nodes and activate the T-helper cells to initiate various immune responses of our body (3).Dendritic cells are classified into lymphoid and myeloid types. The myeloid variants initiate T-helper responses while the lymphoid variants facilitate viral recognition and interferon production (4).

Langerhans cells (LCs) are those highly specialized subsets of DCs that are predominantly present in the epithelial tissues that are lined by stratified squamous epithelium (5). These cells are seen in abundance in the uterus, cervix, epidermal skin and oesophagus. Younes et al., first demonstrated the presence of DCs in the human cervix. After his study, more focus fell on the role of DCs in benign and malignant lesions of the cervix (6). In the uterus, they are present in limited numbers in the surface and glandular epithelium and in endometrial stroma. These cells participate in a pivotal mechanism in initiation of immune responses of host tissue by combating the infections and neoplastic developments in tissues (7). In our study, we have studied the role of CD1a positive DCs in non-cancerous and cancerous tissues of uterine and cervical tissues.

**MATERIALS AND METHODS**

A prospective analysis was conducted in hysterectomy and cervical biopsy specimens over 4 years in a tertiary care teaching hospital in South India where the author was previously employed. Ethical committee clearance from the committee was obtained. Samples were collected from the cervix and body of uterus from women between the age group of 32 and 60 years. These patients had undergone either cervical biopsy for various cervical lesions and hysterectomy for abnormal uterine bleeding or lower abdominal mass or mass descending per vagina.
All patients included in our study had a detailed history of their menstrual cycle. The menstrual cycle staging was done using an idealized 28 day cycle. Endometrium in hysterectomy specimens was further classified according to their menstrual cycle phases as proliferative and secretory phases.

Routine histological processing with Haematoxylin-Eosin staining was done for all samples collected. The processed tissues were embedded in paraffin blocks, labelled and stored in Department of Anatomy. Sections were made from those paraffin blocks, with each section of 3μm thickness. Immunohistochemical staining was done by a technician with more than 10 years of experience. Immunostaining of the sections was done using monoclonal antibodies for anti CD1a. Retrieval of antigen retrieval was performed before immunostaining for all sections taken on charged slides. Peroxidase block was used to neutralize the endogenous peroxidase activity. Then sections were incubated with primary Ab and target binder was used to augment the diffusion of the subsequent polymer reagent. Then sections were processed with substrate/ Chromogen, 3,3 diaminobenzidine (DAB). Reaction of DAB and peroxidase produces brownish precipitate at the antigen site. Sections were counter stained using Hematoxylin stain. Skin was used as a control slide.

The dendritic cells were identified in the cervical and uterine specimens. The number of CD1a positive cells was counted per 20 high power field (40X objective and 10X eyepiece). The average CD1a positive cells per high power field were then subsequently calculated. An adequately sized nucleated cell with visible processes that extend and project from each of the cells were defined as the morphological anatomy of the DCs. All the immune-stained cells were reviewed by 2 independent blinded observers and the photographs were all taken using high definition camera.

SPSS version 16 was used for performing statistical analysis. Descriptive data including mean, median, range and standard deviation were obtained and analysed. Student independent t-test and Fischer’s exact test were used to compare number of cells in proliferative phase, secretory phase, hyperplasia and adenocarcinoma specimens and in the cervical and benign lesions of the cervix.

**RESULTS**

A total of 80 specimens were studied. Hysterectomy and cervical biopsy specimens in the pre and post-menopausal groups were included in our study. Data of all the patients were entered in an Excel sheet, tabulated and analyzed. Table 1 shows the demographic data of all specimens studied. Forty-six uterine and 34 cervix specimens were included in our study.

| Pathological diagnosis (n=80)            | Total number of specimens studied (N=80) | Demographic data (n=80) |
|----------------------------------------|-----------------------------------------|------------------------|
| Adenocarcinoma uterus                  |                                         | Total number of specimens studied (N=80) |
| Squamous metaplasia cervix             |                                         | Total number of specimens studied (N=80) |
| Squamous cell carcinoma cervix         |                                         | Total number of specimens studied (N=80) |
| Non-specific cervicitis                |                                         | Total number of specimens studied (N=80) |
| Cellular atypia (uterus & cervix)      |                                         | Total number of specimens studied (N=80) |
| Proliferative endometrium              |                                         | Total number of specimens studied (N=80) |
| Secretory endometrium                  |                                         | Total number of specimens studied (N=80) |

The minimum age of the patients was 32 and the maximum was 60 years, with a mean of 45.7 years and median of 46. Most patients (n=44) presented with bleeding per vagina. All patients with endometrial carcinoma or squamous cell carcinoma presented with bleeding, while 10 out of 18 patients with non-specific cervicitis also presented with bleeding per vagina. Fibroid uterus was the most common indication for hysterectomy in 35% of cases, followed by adenomyosis in 16% of cases. Endometrial carcinoma of uterus and post menopausal bleeding were the least common causes (6.25% each). The minimum DCS per 20 HPF was 1 and the maximum was 246 with an overall mean of 72.7 DCS per 20 HPF. Table 2 illustrates the number of DCS per HPF in cervical and uterine specimens.

Amongst the cervical specimens, maximum DCS (9.3/HPF) were present in patients with non-specific cervicitis, while the least numbers (5.3/HPF) were present in patients with non-specific cervicitis. The uterine specimens had less number of DCS when compared to cervical tissues. Proliferative and secretory endometrium had maximum DCS while endometrial carcinoma showed

**Table 1: Demographic data of specimens studied**

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| Squamous metaplasia cervix   |                                         | Total number of specimens studied (N=80) |
| Squamous cell carcinoma cervix|                                         | Total number of specimens studied (N=80) |
| Non-specific cervicitis      |                                         | Total number of specimens studied (N=80) |
| Cellular atypia (uterus & cervix) |                                         | Total number of specimens studied (N=80) |
| Proliferative endometrium    |                                         | Total number of specimens studied (N=80) |
| Secretory endometrium        |                                         | Total number of specimens studied (N=80) |

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Amongst the cervical specimens, maximum DCS (9.3/HPF) were present in patients with non-specific cervicitis, while the least numbers (5.3/HPF) were noted in cervical cancers. The uterine specimens had less number of DCS when compared to cervical tissues. Proliferative and secretory endometrium had maximum DCS while endometrial carcinoma showed
the least numbers of DCs. The mean cervical and uterine DCs per HPF were 7.7 and 0.7 respectively. Statistical difference between the two groups was observed. (P, 0.0116).

The dendritic cell distribution in the benign and malignant lesions of uterus and cervix is given in Table 3. Of the 80 specimens, 55 were benign. The remaining 25 included endometrial carcinoma of uterus, carcinoma cervix and dysplasia and metaplasia of the uterus and cervix. The mean DCs per HPF in the benign group was 9.23 and that in the malignant group was 1.762. Though the number of DCs in the benign tissues largely exceeded the numbers in the malignant group, the difference between the two was not statistically significant (p= 0.4961).

Fig. 1 illustrates the mean DC distribution amongst the benign, malignant and atypical cells in uterine and cervical specimens. Maximum DCs were noted in the benign tissue and the DC number was least in the malignant specimens. Fig. 2 illustrates the various CD1a positive cells of uterus and cervix specimens.

### Table 2: Dendritic cells’ distribution in cervical and uterine specimens in high power field

| Specimen studied          | DCs/ HPF | DCs/ HPF | P-value |
|---------------------------|----------|----------|---------|
| Cervix (n=34)             |          |          |         |
| Chronic non-specific cervicitis | 9.3      |          |         |
| Squamous metaplasia/atypia | 6.5      |          | 0.0116  |
| Squamous cell carcinoma    | 5.3      |          |         |
| Uterus (n=46)             |          |          |         |
| Cellular hyperplasia/atypia| 0.1      |          |         |
| Adenocarcinoma             | 0.1      |          |         |
| Proliferative endometrium  | 1.0      |          |         |
| Secretory endometrium      | 0.8      |          |         |

### Table 3: Dendritic cell distribution in benign and malignant specimens

| No of CD1a positive DCs/HPF | Benign/Malignant | Histological type | Mean DCs/ HPF | SD | Min | Max | Benign/ Malignant (DCs/HPF) | p value |
|----------------------------|------------------|-------------------|---------------|----|-----|-----|-----------------------------|---------|
| Benign (n=55)              |                  | Nonspecific cervicitis | 9.30          | 1.67 | 5.8 | 12.3 | 9.23                        |         |
|                            |                  | Proliferative      | 0.95          | 1.68 | 6.7 | 12.5 |                             |         |
|                            |                  | Secretory          | 0.75          | 1.37 | 5.9 | 11.1 |                             |         |
| Malignant (n=25)           |                  | Cervical cellular atypia | 6.5           | 0.65 | 1.6 | 3.5 | 1.762                       | 0.4961  |
|                            |                  | Carcinoma cervix    | 5.30          | 0.55 | 0.8 | 2.6 |                             |         |
|                            |                  | Adeno Ca uterus     | 0.10          | 0.45 | 0.6 | 1.9 |                             |         |
|                            |                  | Uterine atypia      | 0.09          | 0.12 | 1.5 | 1.7 |                             |         |

Fig. 1: CD1a positive DCs in benign, atypical and malignant specimens of uterus and cervix
DISCUSSION

DCs are those specialized cells that present antigen primarily involve in stimulation and modulation of diverse immune responses in mucous-cutaneous surfaces (8). Female reproductive tract, including uterus and cervix also harbour abundant levels of DCs. Based on their developmental pathway, DCs are broadly classified into myeloid and lymphoid types (9). The lymphoid variety, also called as plasmacytoid variant predominantly facilitates production of Type 1 interferon and is primarily involved in recognition of viruses. In comparison, the myeloid variant plays a pivotal role in the initiation of T-cell response (10). In our study, the immunohistochemical marker for immature DCs, the CD1a, is used to focus on the characterisation and pattern of distribution of these myeloid DCs in the female reproductive tract. CD1a is a cell surface related glycoprotein that is related structurally to the major histocompatibility molecules. They regulate the MHC-independent antigen presenting pathways and are most sensitive and specific markers of immature DCs. This study quantifies the number of DCs during the proliferative and secretory phasesof the menstrual cycles in various benign and malignant specimens of cervix and uterus.

The CD1a immature DCs are distributed in increasing densities in the basal layer of the endometrium in both proliferative and secretory phases of the menstrual cycle. The higher number of CD1a DCs in the uterine endometrium indicates that there may be a hormone regulated influx of immature DCs into the uterine endometrium. However, the DC maturation does not appear to be regulated by the hormones.

The DCs of the uterus perform the dual role of immune-suppression and immune-stimulation. The implanted allogenic embryo, that is present in the uterus, is kept isolated and well protected from the belligerent and hostile immune system of the maternal (host) tissue (11). Even in non-pregnant situations, host DCs play the role of local mediators of immunity during menstruation (12). This form of a highly sophisticated immune system of the uterus protects the foetus inside, from varied infections that it is continuously exposed to (13). Various authors have studied the presence of DCs in the secretory and proliferative phases of endometrium and their structure and functions (14). The concentration of CD1a DCs in the uterine endometrium is less in comparison to the levels of natural killer cells and macrophages. However, these smaller numbers are adequate to guard and safeguard the host tissue from foreign antigen, by initiating a strong host immunity (15). These DCs also have the ability to generate matrix metallo-proteinases (MMPs) that plays a crucial role in immunity during tissue breakdown of menstrual cycles (16).

On the other hand, cervical cancer is one of the common cancers that affect women, especially with Human Papilloma Virus (HPV) infections. The viral load of HPV alone may not be sufficient for the initiation or progression of cervical cancers in women (17). Its clinical manifestations also depend upon the host innate and adaptive immunity. Whenever these antigens escape host immunity, the antigen presenting cells (APCs) participate in combating these HPV infected keratinocytes of cervical epithelium. Thus, these APCs mediate a critical part in adaptive immunity of cervical tissues. Several studies have highlighted the role of DCs in the benign and
malignant cervical tissues. The CD1a positive DCs are observed to be concentrated around the epithelial layers of ectocervix. Hayati et al have also studied the role and distribution of CD1a positive DCs in normal and malignant cervical tissues (18). In our study, we observed a higher CD1a positive DC numbers in the ectocervix, mainly in the normal and inflamed cervical tissues. On the other hand, there was a decrease in numbers of CD1a positive DCs in the cervical cancers. Though the CD1a DC levels of benign tissue clearly exceeded the numbers in malignant tissue, it was not statistically significant. The probable reason for that could be the relatively smaller numbers of DCs in the uterus in comparison to that in cervix. This was evident from the Table 2 data that clearly showed a statistically significant numbers between the cervical and uterine tissues.

Our study draws a parallel with that of Hubert et al, who also observed relatively lower level of CD1a positive DCs in the malignant cervical tissue, in comparison to that in inflammed cervix (19). Kim et al, in their study on the role of CD1a positive DCs in weakened immunity of malignant tissues, observed that the microenvironment in the cancer tissue is thought to be immunosuppressive. Innate and adaptive immunities in such tissues are more often restrained. This correlates well with our observations on cervical cancers (20).

To date, there is no head to head comparison of the distribution of CD1a DCs of cervix and uterus. Ours is probably the first study that illustrates the distribution of these cells in the normal, inflammatory, benign and malignant tissues of the cervix and uterus. A larger sample size comparing and correlating the distribution of CD1a and CD83 DCs in human cervix and uterus would add more relevance and significance to our study.

Limitations of the study

Since very few published literature related to CD1a was available, devising the methodology of the study was a big challenge. The numbers of malignant specimens wereless compared to that of benign lesions. The inclusion of CD83 in the study and comparison with CD1a would make this study more informative.

CONCLUSION

Studies on immunohistochemistry of the presence, pattern and distribution of dendritic cells in the human uterus and ectocervix is very limited. Our study demonstrated a higher concentration of CD1a DCs in the cervical tissue than the uterine specimens. Our study also demonstrated a lesser number of CD1a DCs in the malignant tissue and an increased concentration of these cells in normal and inflammatory tissues, though the difference was not statistically significant.

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DOI: https://doi.org/10.51248/v42i3.1445

Biomedicine- Vol. 42 No. 3: 2022