# The morphology and distribution of dendritic cells in normal and carcinoma cervix

Dissertation submitted for

**M.D Anatomy Branch V** Degree Examination,

**The Tamil Nadu Dr.M.G.R. Medical University**

**Chennai, Tamil Nadu.**

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# DECLARATION

I hereby that the dissertation entitled "The morphology and distribution of dendritic cells in normal and carcinoma cervix" is a bonafide research work done by me under the supervision of Dr. Suganthy J., Professor of Anatomy, Christian Medical College, Vellore, in partial fulfilment of the requirements for the MD Anatomy examination (Branch V) of the Tamilnadu Dr. M.G.R. Medical University, Chennai to be held in April 2015.

Name: Dr. Mohana Priya E.

MD Anatomy (Branch V)

# CERTIFICATE

This is to certify that **"The morphology and distribution of dendritic cells in normal and carcinoma cervix"** is a bonafide work of **Dr. E. Mohana Priya** in partial fulfillment of the requirements for the M.D. Anatomy examination (Branch V) of The Tamil Nadu Dr. M. G. R. Medical University to be held in April 2015.

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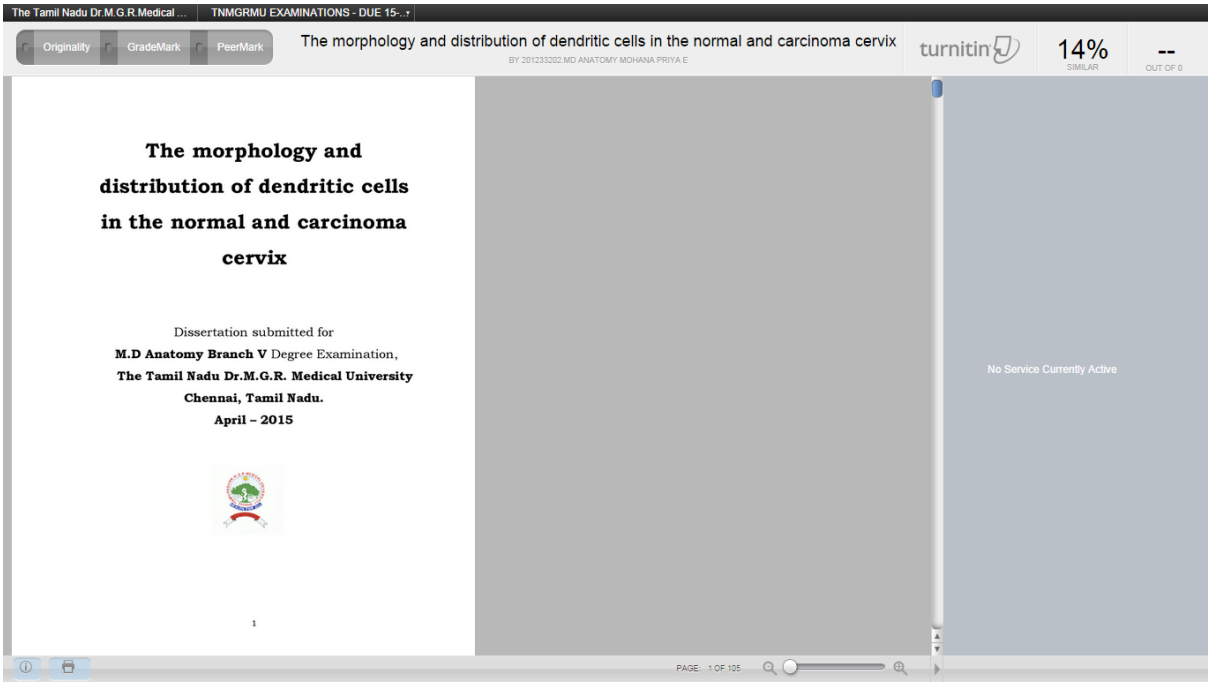
# PLAGIARISM SCREEN SHOT



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# CONTENTS

Title of the abstract :

**THE MORPHOLOGY AND DISTRIBUTION OF DENDRITIC CELLS IN NORMAL AND CARCINOMA CERVIX**

Department : Anatomy

Name of the candidate : Dr. E. Mohana Priya

Degree and subject : M.D, ANATOMY

Name of the guide : Dr. J. Suganthy

**Key words** :

Langerhans cells, CD1a, morphometry and squamous cell carcinoma

**Objectives** :

To study and compare the morphology, size and distribution of CD1a positive Langerhans Cells (LCs) in the ectocervical epithelium of the normal cervix, carcinoma of cervix and normal cervix associated with other genital carcinomas.

**Methods**:

Normal and cancerous ectocervical tissues, obtained after informed consent, from 47 women undergoing hysterectomies, were processed for immunohistochemical staining with CD1a marker and studied by light microscopy. The morphology and distribution of the CD1a positive LCs were studied. The difference in the total number of LCs and different types of CD1a positive LCs per mm length of ectocervical epithelium were compared between the various groups using the Kruskal Wallis test and between pairs of groups using the Mann Whitney U test. The mean diameters of LCs were compared between the groups using ANOVA and pairs of groups using the Scheffe multiple comparison test.

**Results**:

There were significantly fewer CD1a positive LCs in squamous cell and adenocarcinoma of cervix than in normal. An additional previously unclassified variety of LCs having no processes was noted and this was the predominant type in carcinoma cervix. Cells having single processes were significantly higher in normal cervix as compared to cervical carcinomas. The diameters of CD1a positive LCs were significantly higher in normal cervix than in carcinoma cervix. This data may shed new insights into the role of LCs in the disease process of cervical carcinomas.

# 1. INTRODUCTION

Dendritic cells (DCs) are the most potent, professional, antigen presenting cells with the distinctive ability to initiate a primary immune response. They are derived from bone marrow progenitors, which circulate in the peripheral blood and then reach peripheral tissues, where the immature DCs capture invading antigens (1). Hence DCs continuously monitor their microenvironment and when they come across danger signals, they undergo a differentiation and maturation process. Maturing DCs usually migrate to lymphoid tissues where they present antigens to T lymphocyte to commence a primary immune response (2). The maturation of DCs is the important determining factor to begin immunity or immune tolerance (3). After entering DCs, antigens are processed and broken down into peptides in their cytosol and then again expressed on the cell surface of DCs by binding to major histocompatibility complex (MHC) proteins. The peptides bound to MHC class II molecules interact with CD4 positive T helper lymphocytes which results in a profound immune regulatory effect. Similarly, when they bind to MHC class I molecules, cytotoxic CD8 positive T lymphocytes are activated. They recognise and destroy virus infected cells and cancer cells (4). Thus DCs play a key role in host immune defences and a crucial role in anti-cancer immune responses (5).

Langerhans cells (LCs), a type of DCs are MHC class II expressing bone marrow derived DCs. They mainly present as immature cells on different epithelial tissues to detect invading pathogens or foreign antigens (6). After antigen uptake these cells undergo a maturation process and are capable of initiating primary T lymphocyte mediated immune responses in lymph nodes. These cells are known to be present in the epidermis (7), oral mucosa (8), oesophagus (9), glottis (10), lung (11) tongue (12), larynx (13), cervix (14) and vagina (15). In 1980, Figueroa and Caorsi classified LCs in the ectocervix into 5 types based on the number of processes they had as follows: type I- those having one process, type II- with one process which branched, type III- cells with two processes, type IV- those with three or more processes and type V- cells having three or more processes but with several collaterals (16).

Carcinoma cervix is the second most common carcinoma affecting women worldwide (17). 70% of cases are caused by human papilloma virus (HPV) infections mainly HPV 16 & 18 strains (18). First, HPV infected keratinocytes are eliminated by innate immunity resulting from epithelial residing LCs and macrophages. If keratinocytes escape the innate defence, then cellular immunity takes the role of killing HPV infected cells by inducing a systemic response in regional lymphoid organs or a local immune response by LCs in the cervix. Langerhans cells play an important role in both innate and cellular immunity by acting as antigen presenting cells (19). HPV associated carcinoma cervix

can be treated by a virus specific cytotoxic T lymphocyte response produced by an autologous DCs vaccination (20). In the development of cervical neoplasia, where HPV is confined to the epithelium, the presence and maturation of the LCs are vital for the initiation of a tumoricidal response against its progression towards increasing grades of dysplasia and ultimately invasive cancers (21).

## Role of dendritic cells in carcinoma

In carcinomas, immature DCs regularly monitor the environment for foreign antigens. Once the DCs encounter any antigen, they engulf the same by any one of the following processes, namely, phagocytosis, macropinocytosis and endocytosis. This is followed by maturation of DCs by down-regulation of endocytic activity. Subsequent migration to lymphoid tissue results in efficient presentation of optimally processed antigens to T lymphocytes (22). These specified functions of DCs including endocytosis, migration and antigen presentation by DCs help to maintain adaptive immune responses, including an antitumor response against tumour antigens (23). Cytokines like interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF) that are produced by the tumour, inhibit the maturation of DCs (24). The immature cells present in and around the tumour, lack the co-stimulatory molecules necessary for presentation of tumour cell membrane information to the T lymphocytes. Like infectious pathogens, tumours cannot induce the inflammatory response necessary for activation of DCs. This results in an ineffective immune response and disease progression (25). The

number of tumour infiltrating DCs have been found to be clinically important because patients with more number DC infiltration in the tumours lived longer than those who had few or absent DCs (7).

Although, since the 1980s, there has been a growing interest in the study of the distribution and morphometric analysis of LCs in the uterine cervix (26), there is a paucity of information regarding their distribution and morphology in cervical cancer. By quantifying the LCs in cervical carcinomas, it is hoped that more information about the effects of neoplastic cells on LCs can be revealed.

# 2. AIM AND OBJECTIVES

## Aim:

To study the morphology and distribution of CD1a positive Langerhans cells in normal and carcinoma cervix by immunohistochemistry.

## Objectives:

1. To study the morphology of CD1a positive LCs in the ectocervical epithelium of the normal cervix, carcinoma of cervix and normal cervix associated with other genital carcinomas.
2. To count and compare the number of CD1a positive LCs per unit length of ectocervical epithelium in the categories mentioned above.
3. To count and compare the types of CD1a positive LCs per unit length of ectocervical epithelium in the categories mentioned above.
4. To measure and compare the diameter of CD1a positive LCs present in the ectocervical epithelium in the categories mentioned above.

# 3. LITERATURE REVIEW

## FEMALE GENITAL TRACT

The female genital tract is considered to be a part of the common mucosal immune system (27). The female genital tract can be divided into two compartments, the upper and lower genital tracts. The upper genital tract consists of the uterus and Fallopian tubes which are lined by simple columnar epithelium and ovaries. The lower genital tract includes vagina and vulva which are lined by non-keratinized stratified squamous epithelium (28).

## Cervix

The cervix is cylindrical in shape and measures about 2.5 cm long. The upper third of cervix is called the isthmus. It communicates above with the body of the uterus by the internal os. The isthmus undergoes changes during the menstrual cycle and pregnancy. Based on the projection of external end of cervix into upper vagina, the cervix is divided into supravaginal and vaginal parts (28).

### Histology of cervix

Depending upon the lining epithelium, the cervix can be divided into ectocervix and endocervix. The ectocervix is lined by glycogen rich, non-keratinized stratified squamous epithelium. The ectocervical epithelium is divided into 3 layers each having different cell types.

These are from the basement membrane outwards: (1) basal and parabasal cell layers, (2) intermediate cell layer, and (3) superficial cell layer. The basal cells are responsible for epithelial regeneration and parabasal cells shows mitotic figures with a high nuclear-cytoplasmic ratio. The thick intermediate layers are formed by mature cells containing glycogen. Superficial layers are made up of non-proliferating, large, polygonal cells. These cells represent an end stage in the maturation process (29). The cervical canal, lined by highly folded mucosa of columnar mucus cells, is called endocervix (28). The endocervical mucosa differs from the rest of the uterine endometrium the presence of large, branched, mucus secreting cervical glands (30). This mucosa does not desquamate during menstruation. Under the influence of ovarian hormones during ovulation, these glands secrete a watery fluid to facilitate the migration of sperm in the female genital tract. In the luteal phase, under the influence of progesterone and during pregnancy, this mucus secretion becomes more viscous and forms a mucus plug in the cervical canal. This mucus plug prevents the entry of sperm and microorganisms into the body of uterus (31). The core of the cervix is formed by tough and dense collagen fibres with small amounts of smooth muscle (32). Lysis of these collagen fibres leads to dilation of cervix before parturition (33).

**Transformation zone**

The meeting point of the endocervical canal and ectocervix is called the transformation zone. A transition between the lining

epithelium of the endo and ectocervix happens at this region. In the reproductive age group, under the influence of oestrogen, subcolumnar reserve cells of the transformation zone proliferate and undergo squamous differentiation, thereby replacing existing the endocervical columnar glandular epithelium with squamous epithelium (34). The transformation zone is present at the external os before puberty. After menopause, this region recedes inside the cervical canal due to the lack of oestrogen (28). Since 90% of cervical carcinomas arises from this zone, it is considered to be the most important landmark for cytology and colposcopic examinations (35). In normal conditions, more T lymphocytes and antigen presenting cells are seen in the transformation zone and its cervical adjacent tissues in contrast to few T lymphocytes & antigen presenting cells in the normal vagina (36). This region showed higher levels of immunosuppressive agents like transforming growth factor beta-1(TGF-β1) & interleukin-10 (IL-10). These two agents mediate type-1 T cell responses by interfering with antigen presenting cells, thus making this region more vulnerable for development of cancer (37).

## Diseases of cervix

Diseases of the cervix vary from mild inflammation to dysplastic and carcinomatous changes.

**Metaplasia**

The reserve cells lying deep to the columnar epithelium near the transformation zone are transformed into mature squamous cells in a process called metaplasia. These metaplastic cells are normal cells with no nuclear atypical features and do not turn into cancerous cells. However, but in case of atypical metaplasia, cells with abnormal nuclear changes lead to dysplasia and malignancy. The causes for metaplasia are infection, alteration in pH, hormones and mutagens. The hallmark of malignant potential of this cell is presence of aneuploidy, whereas diploidy or polyploidy are present in benign and reparatory cells (38).

**Dysplasia**

Dysplasia represents alteration of cell morphology and disorderly arranged cells of stratified squamous epithelium with variable size, shape and polarity. Thus dysplastic cells show altered nuclear-cytoplasmic ratios and larger irregular, hyperchromatic nuclei with marginal condensed chromatin and mitotic figures (38).

**Cervical intraepithelial neoplasia (CIN)**

The term cervical intraepithelial neoplasia denotes a continuum of disorders from mild, moderate to severe dysplasia and carcinoma in situ. Depending on the replacement of partial or the full thickness of the stratified squamous epithelium by dysplastic cells basement membrane being intact, cervical intraepithelial neoplasia is classified into three grades as follows:

i. Mild dysplasia (CIN I)
ii. Moderate dysplasia (CIN II)
iii. Severe dysplasia (CIN III) (38)

## Cancer cervix

Carcinoma cervix is the second most common carcinoma affecting women worldwide (17). It is the most common genital cancer (80%) incidence in India and accounts for 15% of all other cancers in women (38).

The most common histological subtype of invasive cervical cancers is squamous cell carcinoma, accounting for 80% of tumours. Other types include adenocarcinoma (15%) while other rare types like adenosquamous carcinoma and neuroendocrine tumours constitute the remaining 5%. All types are caused by highly oncogenic HPV. The peak incidence age for carcinoma cervix is 45 years. There is a prolonged duration of precancerous lesion preceding most of the cancers. This

makes the cytological screening effective in preventing invasive carcinomas (39).

***Squamous cell carcinoma of cervix (SCC)***

Squamous cell carcinoma of cervix is a malignant neoplasm derived from the stratified squamous epithelium lining the ectocervix. According to the modified Broder's system, squamous cell carcinomas of cervix are classified as well differentiated, moderately differentiated or poorly differentiated based on the degree of nuclear pleomorphism, mitotic figures, and keratinisation observed (34). However, a weakness of this system is the poor correlation between grade and prognosis (40). Morphological heterogeneity may present even in the same sample due to variable cell types, differentiation, growth patterns, degree of cytological atypia and keratinisation. With increased mitotic activity tumour cells infiltrate in various forms such as anastomosing tracts, nests, groups, compact or irregular sheets (34). Two types of tumour growth are noted normally, they are fungating (exophytic) and infiltrative types (39).

***Adenocarcinoma of cervix***

Adenocarcinoma of cervix is a malignant neoplasm consisting chiefly of glandular epithelium, with foci of squamous neoplastic cells. Proliferation of endocervical glandular epithelial cells having large, hyperchromatic nuclei and cytoplasm devoid of mucin gives darker appearance to the glands when compared with normal endocervical epithelium (39).

**Risk Factors**

70% of cervical cancers are caused by high risk human papilloma virus (18).

**Human papilloma virus**

Human papilloma virus is the main causative agent for development of squamous cell carcinoma of cervix. Route of transmission is mainly genital skin to skin contact. HPV is classified into mucosa associated low risk and high risk groups. Low risk groups are mainly HPV 6 and11. They induce genital warts and self limiting CIN I, whereas high risk group viruses namely HPV 16 and 18 cause CIN II, III or invasive cancer by suppressing genes like p53 and pRb retinoblastoma genes (35). Initially, HPV infection is eliminated with the help of innate immunity produced by epithelial residing LCs and macrophages. When this fails, cellular immunity plays an important role in controlling HPV infection by the DC derived systemic immune response in lymph nodes (19).

Other risk factors include sexual activity less than 18 years, multiple sexual partners, long term use of oral contraceptive pills, family history of cervical cancer, preinvasive lesions, history of sexually transmitted infections like Chlamydia, genital herpes or HIV, tobacco smoking, immunocompromised conditions like human immunodeficiency virus (HIV), chemotherapeutic or steroidal use drug, history of transplantation, history of maternal intake of diethylstilbestrol, low socioeconomic status leading to poor health care

and diets deficient in beta-carotene, selenium, vitamins A,E,C and folate (41). Tobacco smoking increases the risk of squamous cell carcinoma but not adenocarcinoma (42).

**Carcinoma cervix staging based on TNM and FIGO classification**

| TNM | FIGO | Description |
|---|---|---|
| TX | | Primary tumour cannot be assessed |
| T0 | | No evidence of primary tumour [a] |
| T1 | I | Tumour confined to cervix |
| T1a[b] | IA | Invasive carcinoma diagnosed only by microscopy. Stromal invasion with a maximal depth of 5mm measured from the base of the epithelium and a lateral spread of 7mm or less[c] |
| T1a1 | IA1 | Measured stromal invasion ≤ 3mm in depth and ≤ 7mm lateral spread. |
| T1a2 | IA2 | Measured stromal invasion > 3mm and < 5mm with a lateral spread of 7mm or less[d] |
| T1b | IB | Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a/IA2. |
| T1b1 | IB1 | Clinically visible lesion ≤ 4cm in greatest dimension |
| T1b2 | IB2 | Clinically visible lesion > 4cm in greatest dimension |
| T2 | II | Tumour invades beyond uterus but not to pelvic wall or to lower third of vagina |
| T2a | IIA | Tumour without parametrial invasion |
| T2a1 | IIA1 | Clinically visible lesion ≤ 4cm in greatest dimension |
| T2a2 | IIA2 | Clinically visible lesion > 4cm in greatest dimension |

| | | |
|---|---|---|
| T3 | III | Tumour extends to pelvic wall, involves lower third of vagina, causes hydronephrosis or non-functioning kidney[e] |
| T3a | IIIA | Tumour involves lower third of vagina |
| T3b | IIIB | Tumour extends to pelvic wall, causes hydronephrosis or non-functioning kidney |
| T4 | IVA | Tumour extends beyond true pelvis[f,g] |
| M1 | IVB | Tumour spread to distant organs |
| NI | | Regional |

a FIGO no longer includes stage 0 (Tis).

b All macroscopically visible lesions regardless of superficial invasion are T1b/1B.

c Involvement of vascular space or lymphatic, does not affects the classification.

d Invasion is restricted to a measured stromal invasion with a maximal depth of 5 mm and lateral extension of ≤ 7mm. Depth of invasion ≤ 5mm taken from the base of the epithelium. The depth of invasion should always be reported in millimetres.

e On rectal examination, there is no carcinoma-free space between the tumour and the pelvic wall. All cases with hydronephrosis or non-functioning kidney are included, unless with other known causes.

f Bullous oedema is insufficient to classify a tumour as T4.

g Biopsy-proven involvement of bladder or rectal mucosa according to FIGO (34).

## DENDRITIC CELLS

Dendritic cells are potent antigen presenting cells that elicit immune responses by presenting antigens to naïve T cells (43). In 1973, Steinman and Cohn discovered highly branched, motile cells in mouse splenic tissue under phase-contrast light microscopy and named them dendritic cells (44). These cells were named so because of their surface projection resembling the dendrites of neurons (43).

## Development

The bone marrow, human umbilical cord blood and peripheral mononuclear blood cells are the main sources of progenitor cells (45). Bone marrow derived DCs originate from cluster differentiation 34 (CD34) positive haematopoietic stem cells (46)

## Subsets

Human dendritic cells contain several phenotypically and functionally distinct subpopulations (47).

**I. Based on their stage development: (23)**

1. *Immature DCs*
2. *Mature DCs*

**II. Based on their anatomical location:**

1. *Circulation DCs* – DCs and their precursors comprise 1% of mononuclear cells.

2. *Non-lymphoid tissues DC* derived from blood DC precursors include:
    a. Resident DCs – Langerhans cells
    b. Dermal DCs – in the skin
    c. Interstitial DCs – on organs such as liver, lung, kidney and heart
    d. Mucosal DCs – in the oral cavity, respiratory and digestive tracts (1,48)
3. *Lymphoid DCs*

**III. Based on their origin**

1. *Myeloid/ conventional DCs*- Blood monocytes are stimulated to produce myeloid DCs (MDCs) in the presence of granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4 (49).

    MDCs express CD11c as their classical marker. They induce T helper 1(Th 1) responses (47).
2. *Lymphoid / plasmacytoid DCs*- Plasmacytoid DCs (PDCs) express high level of CD123 (IL-3 receptor α-chain) as their surface marker (50). These PDCs, apart from stimulating Th2 response, also produce the most potent antiviral cytokine, interferon alpha (IFN-α) (47). PDCs are found in the thymic medulla and areas of T lymphocytes and responsible for immune tolerance (45).

**IV. Types of Dendritic cells based on their various forms:**

1. *Langerhans cells*- These have been described in detail below

2. *Veiled cells*- the migrant form of DCs in afferent lymphatics (48).

3. *Interdigitating cells*- the dendritic cells present in the para cortical T lymphocyte regions of lymph nodes. These cells resemble DCs in their morphology (51).

4. *Follicular dendritic cells*- the dendritic cells present in the B cell area of lymphatic follicles are called follicular dendritic cells (1). These cells play an important role in the humoral immunity by inducing B cell proliferation, selection and differentiation (52).

## LANGERHANS CELLS

In 1868, Paul Langerhans first described the cells with dendritic process during analysis of gold chloride stained human epidermal skin. In due course these cells were named Langerhans cells. (53). They are the predominant antigen presenting cells in epithelia especially in areas lined by stratified squamous epithelium like epidermis, oesophagus and cervix (54). They play an important role in host protection by acting as adjuvants and conductors of immune responses against infectious agents and neoplastic changes (55).

## LCs ultrastructure

Under electron microscopy, LCs display indented nuclei and their cytoplasm contains free ribosomes, mitochondria, rough endoplasmic reticulum, golgi apparatus, lysosomes, centrioles and the LC specific Birbeck granules. Tonofilaments and desmosomes are absent (56). The unique feature to confirm LCs under electron microscope is the presence of Birbeck granules (57).

## Birbeck granules

Langerhans cells differ from the rest of the DCs by the presence of rod or tennis racket-shaped Birbeck granules. These membranous granules were first discovered by Birbeck in 1961 (53). Birbeck granules acquire a cross striated appearance due to the peculiar arrangement of electron dense particles within them. Frequently, a rod-shaped structure has a vesicle attached at one end giving the appearance of a tennis racket. These Birbeck granules are found mainly near either the Golgi apparatus or the plasma membrane, but also seen in the other regions of the cytoplasm and in their dendritic processes (58). Langerin is associated with Birbeck granules. Induction of Birbeck granules is a consequence of the antigen-capturing function of Langerin, allowing routing into these organelles and providing access to the antigen processing pathway (59).

## Demonstration of LCs

Inability to demonstrate the LCs by routine eosin and haematoxylin staining procedures resulted in employment of special techniques like histochemistry, enzyme histochemistry, electron microscopy and immunohistochemistry.

1. **Histochemical method**

   Langerhans cells were identified for the first time under light microscopy Paul Langerhans by using gold chloride impregnation method. The zinc iodide- osmium (ZIO) method has been successfully used for the demonstration of LCs under both light and electron microscopy (26). Quinine imine dyes such as methylene and cresol blue have also used, but these dyes are not specific for LCs (60).

2. **Enzyme histochemistry**

   The most commonly used enzyme for this method is adenosine triphosphatase (ATPase) but this enzyme is membrane specific rather than cell specific. This enzyme is present in various tissues like those of the nervous system, kidney, liver, bladder and cells like keratinocytes, melanocytes, blood cells and fibroblasts. Thus because of its non specificity and variability, enzyme histochemistry is rarely used to locate LCs (60).

### 3. Electron microscopy

The gold standard used to confirm the presence of LCs is electron microscopy. This method is used to demonstrate the LC specific Birbeck granules (60).

### 4. Immunohistochemistry

Langerhans cells are identified mostly based on their specific surface antigenic markers. The various immunomarkers used are S-100 protein (61), CD1a, langerin (62), vimentin and HLA-DR (63). Of these, CD1a is the most specific and sensitive marker for immature LCs (64,65).

## CD1a

The cluster differentiation 1 (CD1) family of human antigens has four components namely, CDla, CDlb, CDlc and CD1d which are characteristic of the cortical immature thymocytes (66). Of these CD1a is a cell surface glycoprotein that is structurally related to MHC molecules (64) and was initially named T6 (67,68). LCs express CD1a molecules at exceptionally high levels and it is considered to be the most specific and sensitive marker for LCs (64).

## Functions of Langerhans cells

Dendritic cells are the most effective antigen presenting cells to initiate primary immune responses. Their functions include-

1. Capture and process of antigens at surveillance sites
2. Initiation and regulation of T lymphocyte and B lymphocyte response by migration to lymphoid organ
3. Minimising autoimmune reactions by creating T lymphocyte tolerance to self-antigens (45).

### Antigen capture

The initiation and propagation of immune responses mainly depend on the ability of DCs to capture antigens. Immature DCs are efficient to do this function via pinocytosis, endocytosis by using C type lectin receptors and phagocytosis (45). Immature DCs are also capable of phagocytosing necrotic or apoptotic antigenic particles and cross presenting them via major histocompatibility I to cytotoxic T lymphocytes (69).

### Antigen processing and presentation

Immature DCs, after capturing antigens, convert antigenic proteins into peptides, load them on intracellular major histocompatibility complex I or II, and finally deliver these peptide-MHC complexes to the plasma membrane (Figure 1). Phagocytosed extracellular antigens form peptide-MHC II complexes with

costimulatory molecules and present them to CD4 positive T lymphocytes. The peptide-MHC I complexes bind with CD8 positive T lymphocytes to induce cytotoxic effects (70).

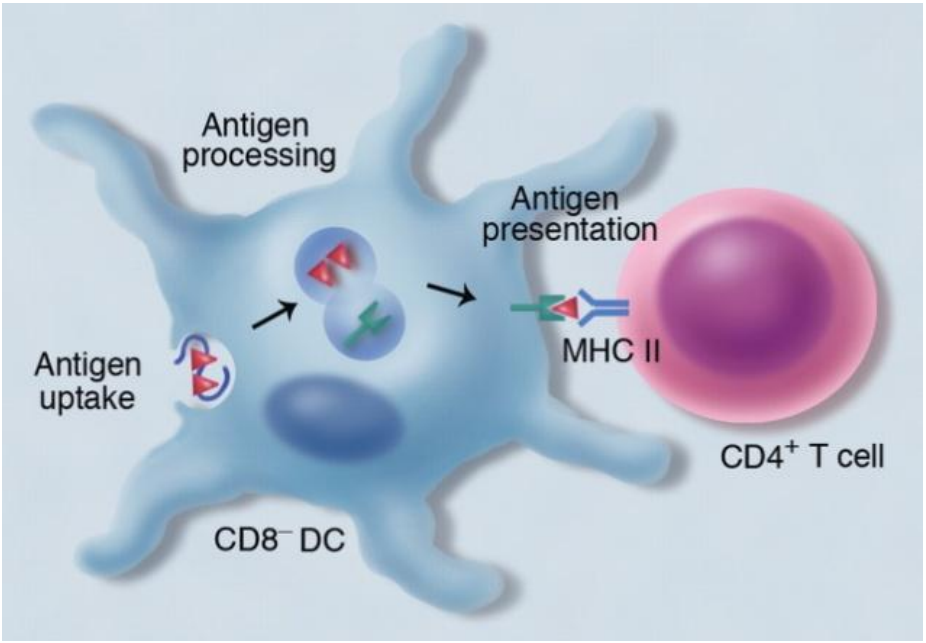



**Figure 1: A schematic picture depicting the process of antigen uptake, processing and presentation by a LC (71)**

**Migration**

DCs, after capturing antigens migrate via blood vessels, afferent lymphatics or high endothelial venules to reach lymphoid organs and present the antigens to lymphocytes for initiation of an immune response (72).

**Maturation**

The maturation process is initiated once the DCs capture antigens and is completed during dendritic cell-lymphocyte interaction (45). Bacteria and their products namely lipopolysaccharides, inflammatory mediators such as tumour necrosis factor-alpha (TNF-α), and interleukin-1 stimulate DC maturation. DCs undergo phenotypical changes during maturation as follows:

- loss of receptors responsible for endocytosis and phagocytosis
- enhancement of costimulatory molecules and chemokine receptor 7 to induce migration
- strong expression of peptide-major histocompatibility I or II complexes (73).

**Apoptosis**

Programmed cell death characterized by activation of an endonuclease enzyme to destroy the nucleus is called apoptosis. Unless DCs receive survival signals from T lymphocytes, they are destroyed rapidly in the T lymphocyte area of lymphoid tissues. Activated T lymphocytes could increase DC lifespan in vivo by regulating their expression of the antiapoptotic Bcl-x molecule (74).

## LANGERHANS CELLS IN THE CERVIX

Younes et al (75) and Hackeman et al (76), demonstrated LCs in the cervix under electron microscopy for the first time in 1968. Hackeman et al., noted a few basal and suprabasal clear cells without desmosome attachments in the normal cervix similar to the morphological features of LCs described earlier (76). Figueroa and Caorsi described the morphology of LCs and measured their density per unit area. This density was much lower than that found in normal human epidermis. They stated that their distribution distribution was uneven but cells appeared to be preferentially located in the vicinity of the external os. They also described their ultrastructure in the normal ectocervix. They proposed that different types of LCs found in the normal human ectoocervix represent different degrees of antigenic stimulation of the cells at the moment of fixation. Those with the largest number of processes, and therefore with the largest surface, might contain a large number of surface receptors and so display a high antigen-binding activity. In normal human ectocervix the most frequent types of LCs were those with few processes, whereas cells with highly branched processes were rare (26).

In 1983, HLA-DR expressing Langerhans-like cells were demonstrated in the ectocervix and vaginal epithelium (15). The presence of LCs in the normal epithelium of the ectocervix and transformation zone was confirmed in the same year (14). Vargas et al.,

in 1989, studied LCs in cervix by using T6 and HLA-DR and these cells were confirmed to be LCs by the presence of Birbeck granules under electron microscopy. They also found that LCs were associated with T lymphocytes both in the epithelium and in the lamina propria by using a T8 lymphocyte specific marker. The electron micrographs disclosed that both LCs and T lymphocytes occupy the intercellular channels of the cervical epithelium. It was proposed that in the cervix, LCs function as antigen presenting cells for T lymphocytes in normal physiological conditions (77). Morelli et al., described the density and distribution of LCs in the normal cervix in 1992. He reported that the density of LCs increased towards the basement membrane and the dendritic aspect was more prominent in the superficial layers (78).

It has been reported that in pregnant women and smokers the number of LCs in the normal squamous epithelium of the transformation zone is less (79). Other authors reported decreased number of OKT6 positive LCs in cervix with HPV lesions. During follow up it was noted that the number of LCs was less in HPV progressed cases when compared to HPV persistent lesions. This study highlighted the role of dendritic cells against viral infections (80). One study analysed MDC and PDC populations in the cervical mucosa and peripheral blood of Chlamydia positive women. In their study, healthy controls had significantly lower numbers of MDCs and PDCs in their cervical sample as compared to Chlamydia positive groups (81).

A study of CD1a positive LCs in different cervical conditions revealed the presence of a relatively high number of LCs in both normal ectocervix and mature squamous metaplastic epithelium, high number of LCs in subcolumnar reserve cell hyperplasia and immature squamous metaplasia but a low number of LCs in endocervical columnar epithelium and in cases of dysplasia (82). A decreased number of LCs was noted in CIN I lesions and it was proposed that there was a decrease in epithelial cell-mediated immune response in women with low grade CIN (79). Others reported an increase in the more ramified types of LCs in cervical intraepithelial neoplasia and invasive carcinomas (83,84).

A study quantifying S-100 positive LCs in invasive squamous cell carcinoma of cervix and interdigitating, follicular dendritic cells in obturator lymph nodes showed that the number of LCs in situ in infiltrating carcinoma increased significantly as compared with control groups. There was no change in LC numbers between grades II and III of squamous cell carcinoma. The results suggested that progression of cervical carcinoma was closely related to decrease in LCs in situ and interdigitating dendritic cells in the regional lymph nodes (54).

## LCs in carcinomas

Langerhans cells play a vital role in the primary immune response to carcinoma. Tumour-infiltrating LCs have been found to be clinically significant in many human malignancies such as carcinomas of the colon, stomach, lung, breast, liver and gall bladder (85). The

function of LCs in tumour conditions includes the following steps in order:

a. Immature LCs identify and capture tumour antigens by endocytosis.

b. Activated LCs convert into their mature form, with loss of endocytotic activity. This is accompanied by accumulation of non-specific cells like eosinophils, natural killer cells and macrophages.

c. By up-regulating MHC complexes, LCs present the processed tumour antigens to CD4 and CD8 positive T lymphocytes.

d. Antigen specific T lymphocytes are activated and proliferate.

e. Finally, these lymphocytes migrate towards the tumour site to kill the transformed cells.

This mechanism is responsible for spontaneous regression of human cancers in some people by maintaining adaptive immunity for anti-tumour response (86). Alteration in any one of these steps can lead to tumour progression. Apart from infections other factors like stress, therapeutic treatment by chemo and radiotherapy, and surgical procedures procedures prevent LC differentiation and activation in cancer patients by producing glucocorticoids, catecholamines, cytokines & chemokines (55).

Although LCs normally have anti-tumour activity, in cancerous lesions they are malfunctioned and polarized into immunosuppressive or tolerogenic regulatory LCs, resulting in tumour progression (87).

Tumour induced inhibition of IL-6, macrophage colony stimulating factor mediated LC differentiation from monocytes and stimulation of monocyte differentiation into macrophages affects the antigen presenting function of LCs. Thus tumours can prevent priming of tumour-specific T lymphocytes by LCs (24).

Tumour derived factors like IL-10 and VEGF help in tumour progression by inhibiting LC maturation (24,45). The immature LCs in and around the tumours lack the expression of the costimulatory molecules which are essential to hand over information of the tumour cell membrane to T lymphocytes. Unlike infectious pathogens, tumours fail to induce an effective inflammatory response to optimize LC activation resulting in a weak immune response (25)

In cancer patients there is uptake of extracellular lipids and this gets accumulated inside the DCs. These lipid laden LCs decline in their capacity to process encountered tumour antigens or to induce naïve T cells but their capacity to express major histocompatibility complex and co-stimulatory products is not affected (88). DCs/LCs induce cytotoxic effect against HPV 16 envelop proteins E6 & E7 and target cervical epithelial cells. This effect is mediated by the pro-inflammatory cytokine molecule IFN-gamma, stimulating the expression of Tumour necrosis

factor-related apoptosis inducing ligand (TRAIL) on target cervical epithelial cells (89). Dysfunction of LCs in patients with cancer is due to accumulation of immature cells and reduction in mature competent LCs, especially in the blood and lymph node. This effect is mainly produced by pooling of myeloid derived immature cells in different stages of differentiation (36).

## LCs in other squamous cell carcinomas

An increased number of LCs has been reported in benign epidermal tumours (90). However contradictory findings regarding the presence and the number of LC have been reported in squamous cell carcinomas both of cutaneous and mucosal origins (7,90–92).

### Oral carcinoma

A comparative study of the number of LCs in normal oral epithelium, oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) revealed less LCs in increasing grades of both OED & OSCC groups when compared with the normal. More LCs were found predominantly in the basal and suprabasal regions in OED but towards the surface in OSCC. In all grades of OSCC, there were less LCs in the subepithelial area. This could be due to migration of cells towards tumour stroma. The advanced cases of poorly differentiated OSCC showed very few LCs in the stroma as well as overlying epithelium above it (8).

**Squamous cell carcinoma of oesophagus**

A study investigating HLA-DR and S-100 positive LCs in 59 human oesophageal squamous cell carcinomas showed a dense infiltration of both type of LCs was detected in 11, only HLA-DR positive LCs in two, and only S100 positive LCs in one specimen. In the remaining 45 tumours infiltrating LCs were sparse, groups with dense infiltration of LCs had better survival rates compared to those with sparse infiltration (9). In another study by the same author on the distribution of HLA-DR positive LCs and tumour infiltrating lymphocytes in 67 patients with squamous cell carcinoma of the oesophagus, HLA-DR positive LCs were chiefly present among the cancer cell nests, and a few were noted in the stroma. T lymphocytes were detected as clusters in the tissue around the cancer cell nests and as individual cells within the tumour nests. T lymphocytes in the tumour nests showed direct contact with HLA-DR positive LCs. A correlation was demonstrated between the number of HLA-DR positive LCs and T lymphocytes in each patient. In addition, dense tumour infiltration by both LCs and T lymphocytes was associated with a better prognosis (93).

**Squamous cell carcinoma of tongue**

A retrospective study of 43 case of primary squamous cell carcinoma of tongue was conducted to determine whether survival and recurrence rates correlate with the degree of LCs infiltration in the

primary tumour and in tissues adjacent to the tongue. In addition, relation between tumour and nodal stage with LCs infiltration was studied. Patients who had greater numbers of CD1a positive LCs adjacent to the tumour had better survival and decreased recurrence rates. The other subpopulations of LCs examined were not associated with survival or recurrence. In addition, the number of CD1a positive LCs in the peritumoral epithelium decreased as the tumour stage increased and if nodal metastases were present. Results of this study showed the association between CD1a positive peritumoral LCs and patient outcome, suggesting an important role of CD1a positive LC populations in squamous cell carcinoma of tongue (12).

**Squamous cell carcinoma of glottis**

CD1a positive and S100 positive LCs in 111 specimens of glottic squamous cell carcinoma were studied retrospectively. Correlations of CD1a positive LCs to pathologic degree, T stage, local recurrence and prognosis were analyzed. Positive rates of CD1a positive LCs were observed in well differentiated, T1-T2 stage and non recurrent groups when compared to moderate and poorly differentiated groups, T3-T4 stage and groups with local recurrence. High expression of LCs was associated with better prognosis (10,94).

**Laryngeal carcinoma**

S-100 positive and CDla positive interdigitating cells were found in the peritumoral infiltrate of 18 patients with squamous cell

carcinoma of the larynx. Under electron microscopy, these cells had intimate contacts with lymphocytes. They played a role in setting up a T-cell immune reaction against neoplastic cells (95). CD1a positive LCs were studied in 50 primary laryngeal squamous cell carcinomas, 10 specimens with metastasis and three samples with recurrence samples. Although statistical associations between number of LCs and clinical-pathological parameters or survival were not found in this study, the number of LCs was increased in patients with marked infiltration of lymphocytes, mainly cytotoxic T cells (13).

**Squamous cell carcinoma of skin**

A marked increase was found in the number of LCs in seborrheic keratoses, an epidermal benign condition, studied by transmission electron microscopy (90). The number and morphology of LCs were investigated in the epidermal squamous cell carcinomas of sun-exposed skin in 10 patients using ATPase as a marker. There was no apparent difference between the numbers of LCs in sun-exposed vs. unexposed skin from the same individual. A significant decrease in the number of LCs was observed. These LCs exhibited round or oval rather than highly dendritic morphology (7).

## LCs in other tissues with adenocarcinoma

### Breast Carcinoma

The presence of immature and mature LCs was shown in 32 specimens with adenocarcinoma of breast adenocarcinoma specimens. In all the specimens, CD1a positive immature LCs were retained in the tumour area whereas CD83 positive mature cells were located in the peritumoral area in 20 specimens (96). A study of the number of CD1a positive LCs within the lymphoid infiltrate of fifty two ductal breast carcinoma specimens, noted the number of LCs was less in comparison with other cells like macrophages and T cells in most cases (97). Others reported that both LC numbers and dendritic cell activation were deficient in human breast cancers, regardless of histological grade of tumour (98).

### Lung Carcinoma

LCs were demonstrated by using S-100 marker in 90 lung carcinomas. S-100 positive LCs were observed more frequently in type II alveolar cell type compared to goblet cell type adenocarcinomas. In addition, small numbers of LCs were noted in a few squamous cell carcinoma & large cell carcinomas (99). A study of 46 specimens of lung adenocarcinomas using S-100 as a marker showed high LCs infiltration in19 specimens with slight infiltration of LCs in the remaining 27. The 5 year survival rates of these two groups were calculated as 61% & 21% respectively. This study concluded that the prognosis of lung

adenocarcinoma is better in the markedly infiltrated LCs group when compared to the slightly infiltrated group (100)

**Colorectal carcinoma**

CD83 and CD1a positive LCs were studied in twenty colorectal adenocarcinoma specimens. It was noted that CD83 positive mature LCs were presented as clusters along the invasive tumour stromal margin whereas CD1a positive cells were present scattered rather than in clusters, all over the tumour tissue. However their numbers were the same as CD83 positive cells in the tumour margin (101). It was noted that the infiltration of more than 30 S100 positive LCs in 10 high power fields showed better prognosis when compared to cases with lower infiltration, without metastasis (102).

**Gastric carcinoma**

S-100 positive LCs and anti-lysozyme antibodies were demonstrated in 174 cases of gastric carcinoma. LCs were mainly interspersed among the tumour cells, whereas macrophages were present in the stroma and around the necrotic foci. Cases with a marked infiltration of LCs, had longer survival time when compared with cases with slight infiltration (103).

**Thyroid carcinoma**

It was observed that in the thyroid gland, compared to follicular carcinomas, papillary carcinomas contained marked infiltration of

CD1a positive LCs, IL-1α and TNF-α positive cells. More LCs were noted in tumour areas and interdigitating cells were seen around the tumour areas along with LCs. Cancer cells producing IL-1α and TNF-α controlled the infiltration and maturation of LCs. This process was maintained in papillary carcinoma in contrast to follicular carcinoma. This study revealed that thyroid carcinomas with dense infiltrations of LCs were correlated with a favourable prognosis (69).

The above studies reveal that, LCs present in tumour areas of both squamous and adenocarcinomas of different tissues is directly associated with prognosis.

## HIGH ENDOTHELIAL VENULES AND LCS

High endothelial venules (HEVs) are specialized postcapillary venules lined by cuboidal endothelial cells, bulging into the lumen. HEVs are present in secondary lymphoid organs, except the spleen (104). DCs have been seen around the walls of HEVs and are important for maintaining HEVs characteristics (105). HEVs control the extravasation of naive lymphocytes from the blood into the afferent lymphatics through which they reach the secondary lymphoid organs (106). This migration of naïve T and B lymphocytes through the HEVs is limited by LCs especially CD11c positive cells. This event is mediated by lymphotoxin derived from the CD11c positive cells (105). Naïve T and B cells migrate through HEVs by the following steps namely rolling, sticking, crawling and transmigration. Finally, naïve T and B

lymphocytes reach the paracortex and lymphatic follicles respectively (104). Solid tumours like breast, colon, lung, ovarian carcinomas and melanomas contain vessels similar to HEVs in their stroma. The tumour induced HEVs were associated with increased level of B and T lymphocyte infiltration and good prognosis carcinoma of breast (104).

# 4. MATERIALS AND METHODS

## 4.1 COLLECTION OF SPECIMENS

Ethical approval from the Institutional Review Board, Christian Medical College, and Vellore was obtained for the study.

**Sample size estimation**

The sample size calculation was done based on data available from a previous study (Hayati and Zulkarnean, 2007: Mean in normal group = 3.89 ± 2.63 and squamous cell carcinoma mean = 2.32 ± 1.74) (21). The required sample size to show a difference of 2 units in CD1a positive Langerhans cells between normal and squamous cell carcinoma with 80% power and 5% alpha error was found to be 35 specimens in each group.

| | |
|---|---|
| Standard deviation in group I | 3 |
| Standard deviation in group II | 3 |
| Mean difference | 2 |
| Effect size | 0.666667 |
| Alpha error (%) | 5 |
| Power (1- beta) % | 80 |
| 1 or 2 sided | 2 |
| Required sample size per group | 35 |

Formula:

$$n = \frac{(Z_{\alpha}+Z_{1-\beta})^2 \times 2 \times S^2}{d^2}$$

S- Standard deviation, d- 2, $Z_{\alpha}$- 5% level of significance,

$Z_{1-\beta}$- 80% power of the study

**Inclusion criteria**

- Women undergoing total abdominal hysterectomy in OG I unit for conditions like fibroid uterus, endometriosis, adenomyosis, carcinoma cervix, carcinoma endometrium and ovarian carcinomas.

**Exclusion criteria**

- Immunocompromised patients (e.g. HIV)
- Patients who have undergone chemotherapy and radiation
- Patients unwilling to participate in this study

**Specimen collection**

Cervix specimens were collected from patients who underwent total abdominal hysterectomy, either from the Department of Obstetrics and Gynaecology or from the Department of Pathology, Christian Medical College, Vellore. Cervix with intact epithelium of about 1-2 cm was collected in neutral formalin. In cases of cancer cervix, for the quantitative analysis, tissue away from the tumour area was used. The consent was taken from the participated patients in this study, a day before surgery in the gynaecology ward.

## 4.2 GROUPING OF COLLECTED SPECIMENS

The ectocervix specimens collected were categorized into five groups based on their histopathological diagnosis.

i. Normal cervix – 22
ii. Cervical Intraepithelial Neoplasia I – 2
iii. Squamous cell carcinoma of cervix – 11
iv. Adenocarcinoma of cervix – 4
v. Normal cervix associated with other genital carcinomas – 8

## 4.3 FIXATION AND PROCESSING OF TISSUES

Trimmed cervical specimens were fixed in neural formalin for about 7- 10 days to prevent autolysis. Excess formalin was removed by placing the fixed tissues under running water. Tissues were dehydrated by using ascending grades of isopropyl alcohol (70%, 80%, 90%, 95% and 100%) followed by clearing in toluene, impregnation with molten paraffin wax and embedding in paraffin.

## 4.4 SECTIONING

Five micron thick serial sections were taken using a rotary microtome. The serial sections were allowed to float in the hot water bath at 45°C and mounted on pre-cleaned slides coated with poly-L-lysine (PLL). Slides were air dried, incubated at 37°C overnight and stored in clean slide boxes for staining.

## 4.5 STAINING

### Haematoxylin and eosin staining

Representative sections at regular intervals were stained by the standard haematoxylin and eosin (H & E) staining technique.

### Immunohistochemical staining

Two slides from every specimen, each with 4-5 sections chosen carefully without folds, were selected for immunohistochemical staining. These were incubated in a 57°C oven overnight, the night prior to staining. While staining each set of slides, one slide known to contain CD1a positive LCs was also stained as positive control. Not more than 10 slides were stained in each session.

**Polymer-horseradish peroxidase (HRP) detection system**

A modification of standard avidin-biotin peroxidase technique known as polymer-HRP detection system was used in this study. This biotin free detection system was used to eliminate the problems of endogenous biotin.

I. The Universal HRP Detection system ready-to-use kit was purchased from SCYTEK Laboratories, USA. This pack includes the following reagents:

1. Peroxide block: 3% hydrogen peroxide in water. This was used to reduce nonspecific background staining due to endogenous peroxidises.
2. Super block reagent: 0.25% casein (hydrophilic protein) in Phosphate Buffered Saline (PBS) containing stabilizing protein and 0.015mol/L sodium azide. This is a unique serum-free protein blocking agent.
3. Anti polyvalent HRP polymer: This utilizes a polymerized HRP label that eliminates biotin and its associated background issues from the equation. Effective with antibodies of mouse, rat, rabbit and guinea pig.
4. Diaminobenzidine (DAB) chromogen concentrate in the presence of the peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol.
5. DAB substrate: Tris buffer containing peroxides and stabilizers.

II. The primary antibody used was the monoclonal mouse anti-human CD1a (DAKO, USA)

**Principle of the HRP detection system**

The demonstration of CD1a antigen in tissues by immunohistochemistry method includes two steps

Step 1 – binding of antibody with antigen of interest in specific tissues.

Step 2 – by using various enzyme chromogenic systems the above bound antibody is visualized.

***Solutions prepared:***

I. Two buffers were prepared namely

1. EDTA buffer (pH 9.0) – used during antigen retrieval stage
2. TRIS buffered saline (pH 7.6) – used as wash buffer

For staining 20 slides, the following quantity of buffer solutions were prepared

1. EDTA buffer (1litre)

   a. Tri hydroxy methyl methylamine (Tris) – 6.05 grams

   b. Ethylene diamine tetra acetic acid (EDTA) – 0.7465grams

   c. Distilled water – 1 litre

   The above solutions were mixed well with a glass stir rod and the pH was adjusted to 9.0

2. Tris buffered saline (TBS – 2 litres)

   a. Sodium chloride – 16 grams

   b. Tris – 1.210grams

   c. 1 normal hydrochloric acid – 8 ml

   d. Distilled water – 2 litres

   The above solutions were mixed well with a glass rod and the pH was adjusted to 7.6

II. Preparation of Harri's Haematoxylin

Distilled water – 200ml

Harri's haematoxylin powder – 1 gram

Absolute isopropyl alcohol – 10ml

Potassium alum – 20g

Mercuric oxide – 0.5gram

Powerful nuclear stain Harri's haematoxylin powder was dissolved in absolute isopropyl alcohol by using pestle and mortar. In the florence flask, first the alum was dissolved in distilled water followed by adding the solutions of dissolved Harri's Haematoxylin and mixed well. The flask was heated on a coil stove and then mercuric oxide was added slowly into it. Once the solution became dark purple it was off the heat and allowed to cool. The solution was filtered before each staining.

***Staining method:***

1. Selected slides were taken from 57°C incubator and then deparaffinised in xylene for 30 minutes.
2. The slides were immersed in absolute isopropyl alcohol, for 2 changes of 1 minute each to remove the xylene.
3. Slides were washed for 10 minutes in running water to remove the alcohol.
4. **Antigen retrieval**: The antigenic epitopes are masked by formalin cross links formed during the processes of fixation and embedding. Different primary antibodies have different techniques of antigen retrieval. The heat retrieval method was used for CD1a antigen retrieval in this study. Steps used were as follows:

    a. A trough with EDTA buffer was preheated by heating in a pressure cooker without weight until steam emerged.

    b. Meanwhile the slides were transferred from running water to distilled water for 1-2 minutes. They were then arranged adequately spaced, in a slide rack and transferred to the

preheated EDTA buffer. The cooker lid was closed, the cooker weight valve was applied and returned to the heat.

c. After two whistled (approximately 10 minutes) the cooker was taken off, the stove and kept inside a sink filled with water for rapid cooling. The slides in which sections hadn't floated were selected and transferred into distilled water for 5 minutes.

d. The slides were then washed in TBS twice, each time for 5 minutes.

The remaining steps were carried out in an air conditioned room

5. The slides were arranged on a humidified chamber.
6. **Peroxidase blocking** solution was applied over each section for 10 minutes to block endogenous Peroxidase. This was conducted in a dark room to avoid decomposition of the hydrogen peroxide in the solution.
7. The excess solution was drained from each slide and slides were rearranged in the slide rack. The slides were dipped in 2 changes of distilled water and then placed in TBS for 15 minutes.
8. Excess solutions was wiped from each slide around the tissue sections and then **super block** was applied over each tissue section and returned to the humidification chamber for 10 minutes, to block the endogenous proteins.
9. Excess super block was drained and **primary antibody** was applied over each section using a micropipette and slides were placed inside the humidification chamber for 1 hour.

10. Excess primary antibody was drained and slides were washed in TBS, 2 changes each for 5 minutes.
11. **Enzyme labelled secondary antibody** was applied over each section and kept inside the humidification chamber for 30 minutes.
12. Excess antibody solution was drained and washed in TBS, 2 changes, each for 5 minutes.
13. Meanwhile DAB working solution was prepared freshly by adding 1 drop of DAB chromogen to 1ml of DAB substrate and it was mixed well.
14. **DAB working solution** was added to each section, in a dark room to prevent decomposition of hydrogen peroxide, and kept in the humidification chamber for 5-8 minutes.
15. Excess DAB solution was drained; the slides were washed in TBS for 5 minutes and then placed under running water for 10 minutes.
16. To stain the nuclei of each cell, all slides were counterstained by 2 dips in freshly prepared **Harri's alum Haematoxylin**. Excess stain was removed by washing in tap water.
17. The slides were dipped in lithium carbonate solution for blueing effect and then kept under running water for five minutes.
18. Finally slides were air dried, dipped in xylene and mounted with mixture of distyrene plasticizer and xylene (DPX).

## 4.6 ANALYSIS

### Qualitative analysis

The stained slides were examined under the light microscope Olympus BX43 and photos were taken by using Olympus DP21 camera fitted on light microscope. The presence and morphology of the epithelium was confirmed by studying the H & E stained slides. The test of the study was conducted in the slides stained with CD1a. The morphology of CD1a positive Langerhans cells present in the mucosa of the ectocervix was noted.

### Quantitative analysis

Cellsens standard image analyzing software (version 1.4) was used for the morphometric analysis. The number of CD1a positive LCs was counted and their diameters measured under 40x magnification using Cellsens analysing software. There were 5 groups of specimens collected namely, normal cervix, CIN I, squamous cell carcinoma, adenocarcinoma of cervix and normal cervix associated with other genital carcinomas. The number of CD1a positive LCs per 25mm length of ectocervical epithelium was counted for each specimen and the data classified into the five categories mentioned above. The length of epithelium was measured along the basement membrane using the image analysing software (Figure 2). Only the cells with well defined

nuclei were counted. LCs were categorized into six types, the five types as mentioned in the Figueroa and Caorsi classification (26) and an additional type of cells without processes. The average number of each type of LC and total number were calculated per mm length of epithelium. The diameters of the LCs were taken in two perpendicular axes (Figure 3). The diameters of hundred LCs per specimen with well defined nuclei were measured in micrometers. The average diameters of the cells were calculated.

## Statistical analysis

The data was analysed using SPSS version 16. The descriptive statistics (range, mean and standard deviation) were obtained. The difference in the total number of LCs and different types of CD1a positive LCs per mm length of ectocervical epithelium were compared between the five groups using the Kruskal-Wallis test and post hoc test between pairs of groups using the Mann-Whitney U test was done at 1% level. The mean diameter of LCs were compared between the five groups by ANOVA and post hoc test between pairs of groups using the Scheffe multiple comparison test was done at 1% level.

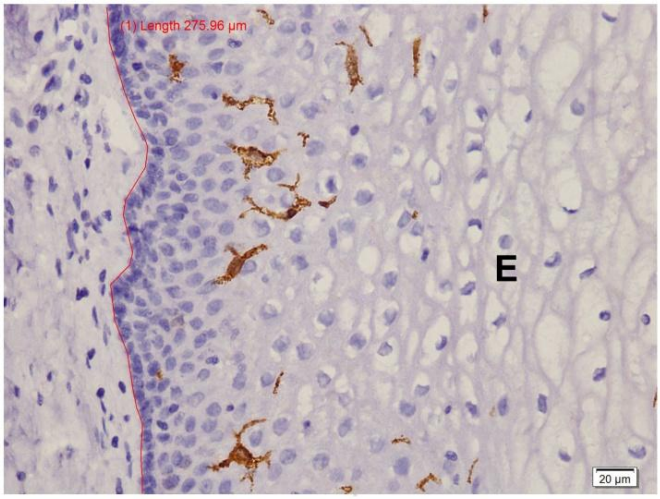



Figure 2: Red line drawn along the basement membrane of the ectocervical epithelium (E) to show the number of CD1a positive LCs (brown cells) counted per millimeter length of basement membrane.

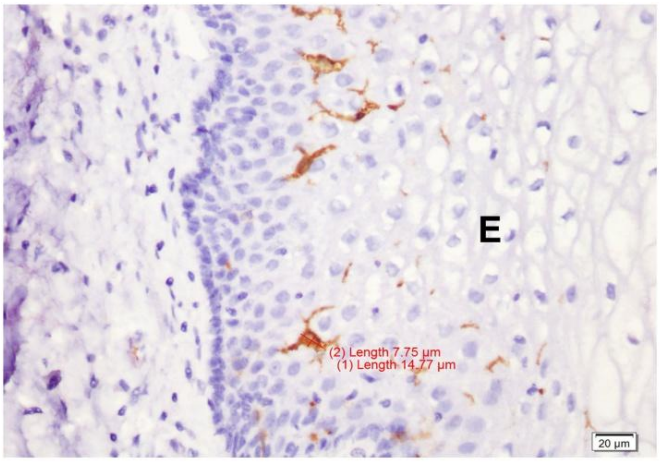



Figure 3: The diameters of LCs were measured in two perpendicular axes (length 1 and length 2) in the ectocervical epithelium (E).

# 5. RESULTS

## Morphology and distribution of CD1a positive LCs in normal and cancerous cervical tissues using immunohistochemistry.

The cervix specimens collected were categorized into five groups based on their histopathological diagnosis namely normal cervix, cervical intraepithelial neoplasia I (CIN I), squamous cell carcinoma of cervix, adenocarcinoma of cervix and normal cervix associated with other genital carcinomas such as carcinoma endometrium and carcinoma ovary.

## Normal cervix

Stratified squamous epithelium lining the ectocervix showed the presence of CD1a positive LCs in all the specimens. Regional variation was present in their distribution. The cells were randomly distributed in the epithelium. While some areas had relatively high density of LCs (Figure 4), some areas were devoid of LCs (Figure 5). These cells showed the classical morphology of dendritic cells. In the epithelium, CD1a positive LCs were present mainly in the suprabasal and midepithelial layers (Figure 4). Few LCs were also noted in the lamina propria of ectocervix (Figure 4). The mean number of CD1a positive LCs per mm length of normal ectocervical epithelium was 8 ± 2.76. The diameter of the CD1a positive LCs ranged from 4.08 to 15.31μm with a mean diameter of 8.57 ± 1.81μm (Table 1).

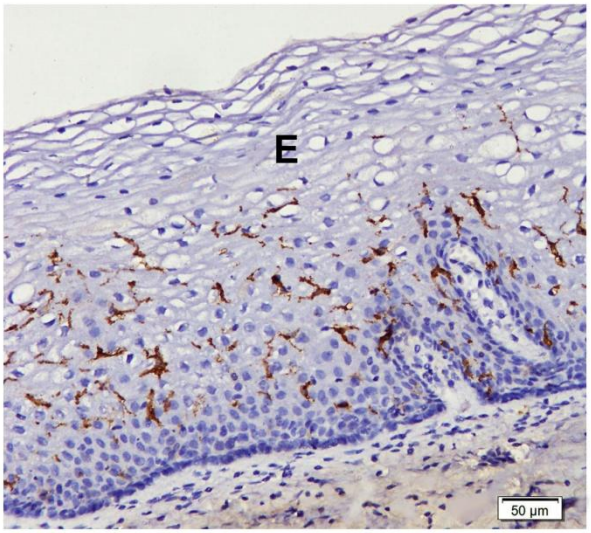



Figure 4: Normal human ectocervical epithelium (E) showing CD1a positive Langerhans cells (brown cells). Note the cells are predominantly present in the suprabasal and mid-epithelial layers.

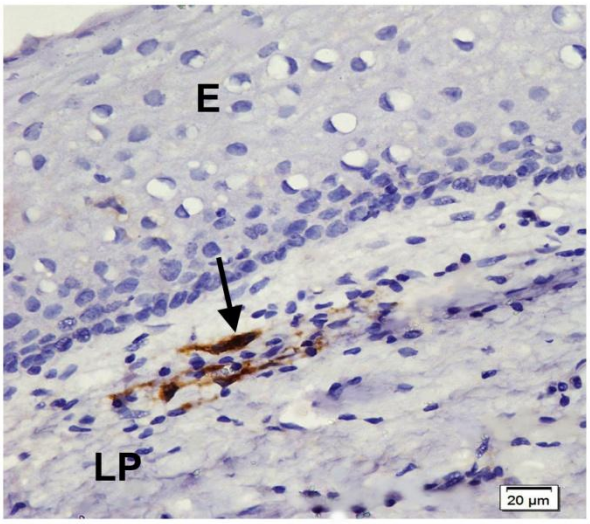
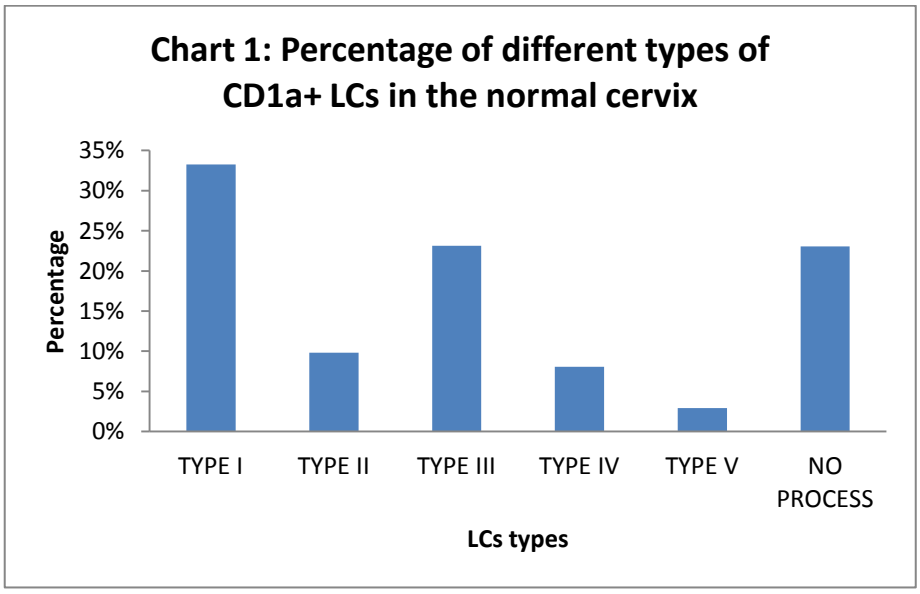


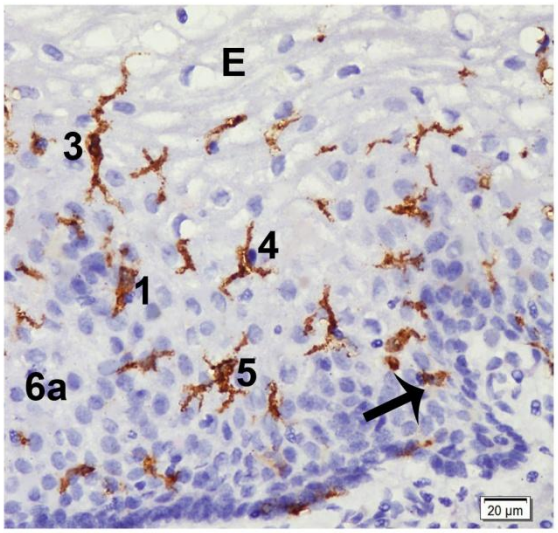

Figure 5: Arrow indicates a CD1a positive LC in the lamina propria (LP) in the normal ectocervix. Note the above epithelium (E) is devoid of LCs.

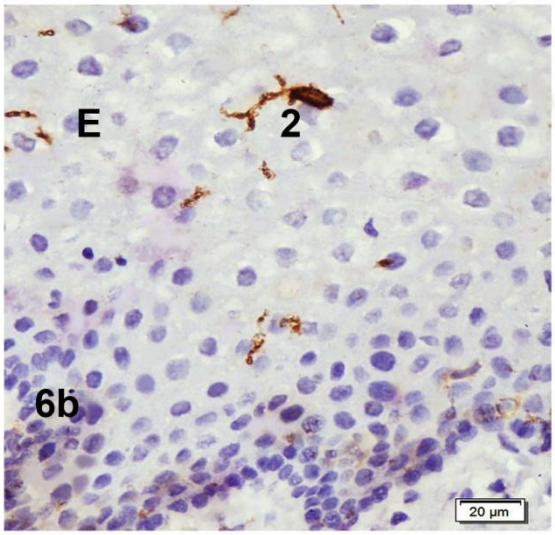
In the epithelium of the normal cervix, all five types of LCs as classified by Figueroa and Caorsi (16) were identified. In addition, CD1a positive cells without processes but having round nuclei were also present (Figures 6a & 6b). Chart 1 & Table 2 show the percentages of each type of LC present in the normal ectocervix. The number of type I LCs was significantly higher than other types of LCs except for the cells without processes ($p < 0.001$) (Table 3). LCs migrating through the basement membrane were also observed (Figure 7).

**Table 1: The number of CD1a positive LCs per mm length of ectocervical epithelium and diameter of cells in the normal cervix**

| Normal cervix | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Number | 8.00 | 2.76 | 3.84 | 16.04 |
| Diameter (μm) | 8.57 | 1.81 | 4.08 | 15.31 |






Figures 6a & 6b: Different types of LCs present in the normal ectocervical epithelium (E). 1- Type I cell with single process, 2- type II cell with single, branched dendrite, 3- type III cell having two processes, 4- type IV cell with three or more processes, 5- type V cell having three or more processes with arborization. Arrow indicates LC without processes which is not included in the Figueroa and Caorsi classification.

**Table 2: Distribution of different types of CD1a positive LCs per mm length of normal ectocervical epithelium and comparison of all the types of LCs using the Kruskal Wallis test.**

| Types of LCs | Mean | Standard deviation | Minimum | Maximum | Percentage | p value |
|---|---|---|---|---|---|---|
| Type I | 2.65 | 0.99 | 1.32 | 4.68 | 33.17% | |
| Type II | 0.78 | 0.65 | 0.12 | 2.40 | 9.79% | |
| Type III | 1.85 | 0.98 | 0.48 | 4.56 | 23.06% | |
| Type IV | 0.64 | 0.45 | 0.16 | 1.80 | 8.04% | **<0.001** |
| Type V | 0.23 | 0.42 | 0.00 | 1.68 | 2.91% | |
| No processes | 1.84 | 1.01 | 0.12 | 3.76 | 23.02% | |

$p < 0.05$ is significant

**Table 3: Comparison between types of LCs within the normal cervix using the Mann Whitney U test**

| S.No | Comparison between types of LCs in the normal cervix | p value |
|---|---|---|
| 1. | Type I vs. type II | **< 0.001** |
| 2. | Type I vs. type III | **0.005** |
| 3. | Type I vs. type IV | **< 0.001** |
| 4. | Type I vs. type V | **< 0.001** |
| 5. | Type I vs. no processes | 0.019 |
| 6. | Type II vs. type III | **< 0.001** |
| 7. | Type II vs. type IV | 0.723 |
| 8. | Type II vs. type V | **< 0.001** |
| 9. | Type II vs. no processes | **< 0.001** |
| 10. | Type III vs. type IV | **< 0.001** |
| 11. | Type III vs. type V | **< 0.001** |
| 12. | Type III vs. no processes | 0.785 |
| 13. | Type IV vs. type V | **< 0.001** |
| 14. | Type IV vs. no processes | **< 0.001** |
| 15. | Type V vs. no processes | **< 0.001** |

$p < 0.01$ is significant

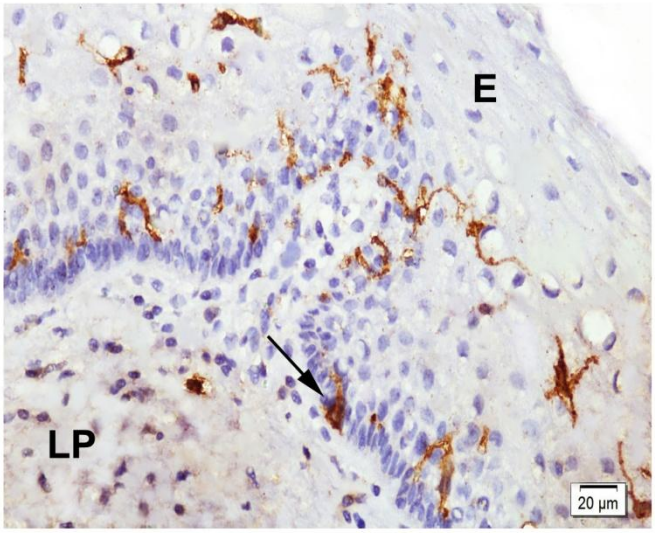



Figure 7: Arrow indicates the migration of a CD1a positive LC through the basement membrane of the stratified squamous epithelium (E) lining the normal ectocervix; LP- lamina propria.

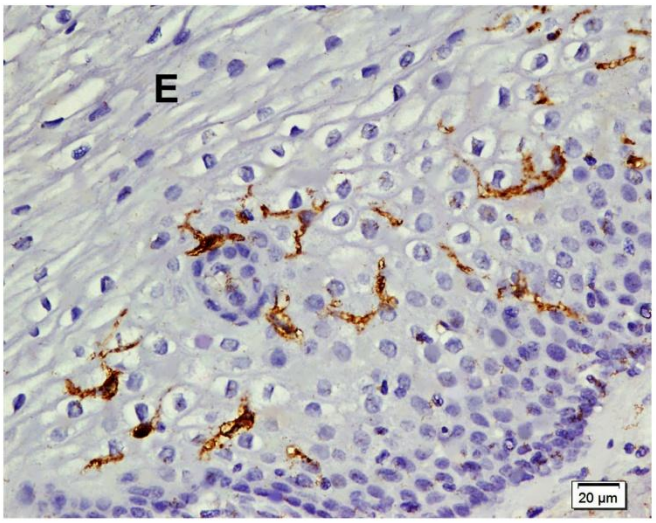



Figure 8: Many CD1a positive LCs (brown cells) with well-defined nuclei and variable numbers of long processes predominantly in the suprabasal layer of ectocervical epithelium (E) in a case of CIN I.

## Cervical intraepithelial neoplasia I

The CD1a positive LCs were predominantly present in the suprabasal layer of the stratified squamous epithelium in the specimens with cervical intraepithelial neoplasia stage I. The mean number of CD1a positive LCs per mm length of stratified squamous epithelium was 10.02 ± 2.01. The diameter of the CD1a positive Langerhans cells ranged from 5.38 to 13.31μm with a mean diameter of 8.75 ± 1.67μm (Table 4). These cells had well defined nuclei and variable number of long processes (Figure 8). The cells with either single or no processes were predominant. There was 35.13% of type I cells and cells with no processes accounted for 33.93% of cells. Cells with three or more processes i.e. type IV and V were decreased in number. There were only 3.99% of type IV and 0.8% of type V cells were present in CIN I ectocervix (Chart 2 and Table 5).

**Table 4: The number of CD1a positive LCS per mm length of ectocervical epithelium and diameter of cells in cervical intraepithelial neoplasia I**

| CIN I | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Number | 10.02 | 2.01 | 8.6 | 11.4 |
| Diameter (μm) | 8.75 | 1.67 | 5.38 | 13.31 |

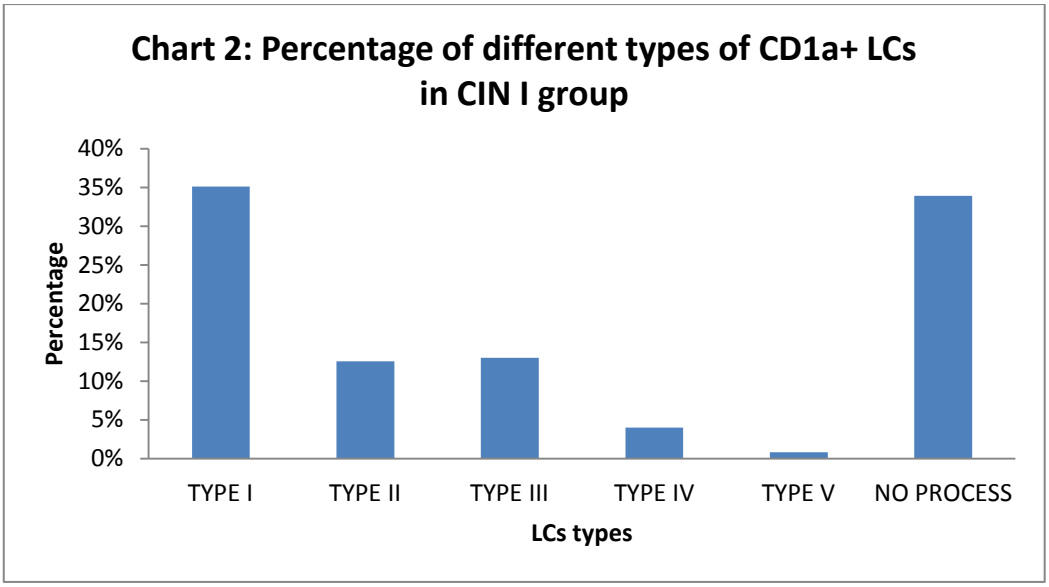



**Table 5: Distribution of different types of CD1a positive LCs per mm length of ectocervical epithelium in CIN I and comparison of all the types of LCs using the Kruskal Wallis test.**

| Types of LCs | Mean | Standard deviation | Minimum | Maximum | Percentage | p value |
|---|---|---|---|---|---|---|
| Type I | 3.52 | 0.17 | 3.40 | 3.64 | 35.13% | |
| Type II | 1.26 | 1.33 | 0.32 | 2.20 | 12.57% | |
| Type III | 1.36 | 0.51 | 1.00 | 1.72 | 13.00% | |
| Type IV | 0.40 | 0.28 | 0.20 | 0.60 | 3.99% | 0.082 |
| Type V | 0.08 | 0.11 | 0.00 | 0.16 | 0.80% | |
| No processes | 3.40 | 1.41 | 2.40 | 4.40 | 33.93% | |

p value < 0.05 is significant

## Squamous cell carcinoma of cervix

In the specimens with squamous cell carcinoma of the ectocervix, CD1a positive LCs were randomly distributed. They were seen either in the entire thickness (Figure 9), or in the suprabasal layer (Figure 10), or in the midepithelium (Figure 11), or infrequently in the superficial layer (Figure 12). The number of CD1a positive LCs was decreased in the epithelium of ectocervix in squamous cell carcinoma. Their mean number per mm length of ectocervical epithelium was 5.36 ± 2.88. The diameter of the CD1a positive Langerhans cells ranged from 3.50 to 17.03μm with a mean diameter of 8.28 ± 2.04μm (Table 6). These were predominantly cells with single process or rounded cells without dendritic processes as described later (Chart 3 and Table 7). The number of type I cells and cells with no processes was significantly higher than type II, type IV and type V cells ($p < 0.001$) (Table 8).

**Table 6: The number of CD1a positive LCs per mm length of ectocervical epithelium and diameter of cells in squamous cell carcinoma of cervix**

| Squamous cell carcinoma of cervix | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Number | 5.36 | 2.88 | 1.64 | 11.48 |
| Diameter (μm) | 8.28 | 2.04 | 3.50 | 17.03 |

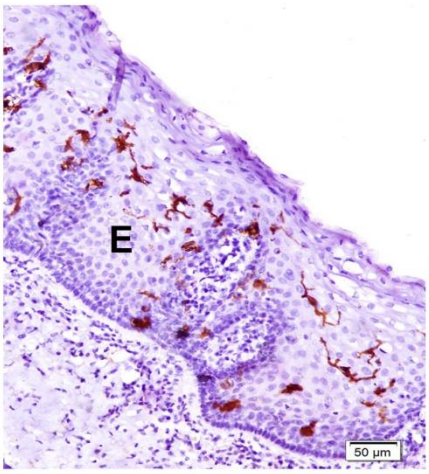



Figure 9: CD1a positive LCs (brown cells) present throughout the ectocervical epithelium (E) in case of moderately differentiated squamous cell carcinoma of cervix with nodal metastasis.

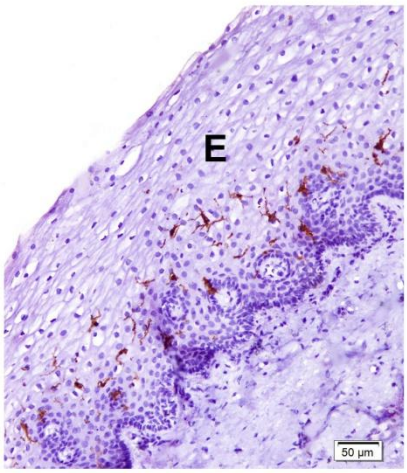



Figure 10: Note the presence of CD1a positive LCs (brown cells) predominantly in suprabasal layers of ectocervical epithelium (E) in a case of squamous cell carcinoma of cervix.

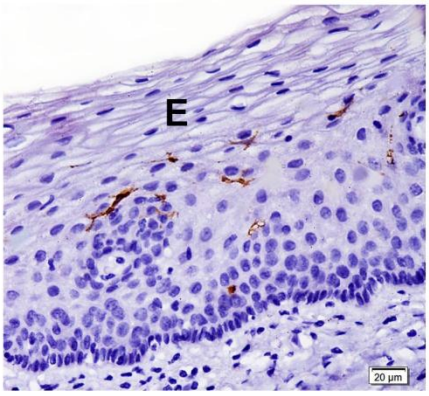



Figure 11: Presence of CD1a positive LCs (brown cells) in the ectocervical mid-epithelial (E) layers in a case of squamous cell carcinoma of cervix.

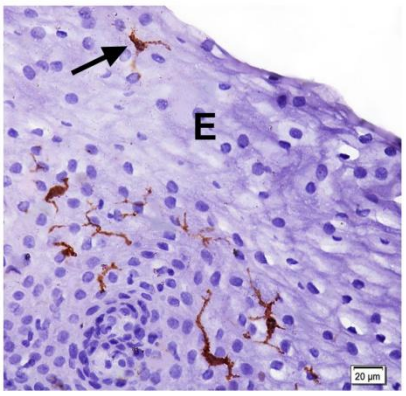



Figure 12: Arrow shows CD1a positive LC in the superficial layers of ectocervical epithelium (E) in a case of squamous cell carcinoma of cervix.

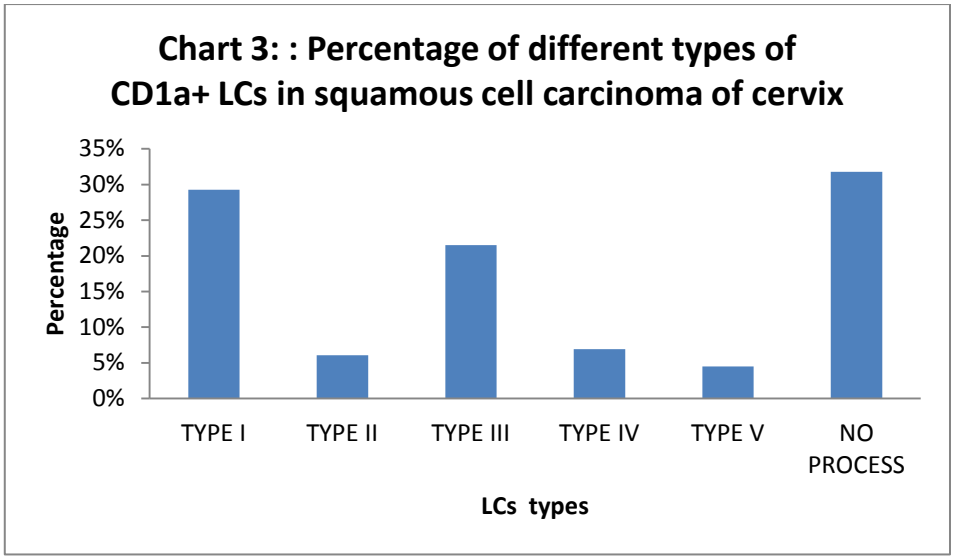



**Table 7: Distribution of different types of CD1a positive LCs per mm length of ectocervical epithelium in squamous cell carcinoma of cervix and comparison of all the types of LCs using the Kruskal Wallis test.**

| Types of LCs | Mean | Standard deviation | Minimum | Maximum | Percentage | p value |
|---|---|---|---|---|---|---|
| Type I | 1.57 | 0.79 | 0.40 | 3.28 | 29.26% | |
| Type II | 0.32 | 0.30 | 0.00 | 0.88 | 6.04% | |
| Type III | 1.15 | 0.94 | 0.04 | 3.08 | 21.52% | |
| Type IV | 0.37 | 0.32 | 0.00 | 1.00 | 6.92% | **< 0.001** |
| Type V | 0.24 | 0.32 | 0.00 | 1.00 | 4.48% | |
| No processes | 1.70 | 0.78 | 1.00 | 3.12 | 31.77% | |

p value < 0.01 is significant

**Table 8: Comparison of the types of LCs within the squamous cell carcinoma of cervix using the Mann Whitney U test**

| S.No | Comparison of the types of LCs in squamous cell carcinoma of cervix | p value |
|---|---|---|
| 1. | Type I vs. type II | **< 0.001** |
| 2. | Type I vs. type III | 0.176 |
| 3. | Type I vs. type IV | **< 0.001** |
| 4. | Type I vs. type V | **< 0.001** |
| 5. | Type I vs. no processes | 0.987 |
| 6. | Type II vs. type III | **0.008** |
| 7. | Type II vs. type IV | 0.735 |
| 8. | Type II vs. type V | 0.370 |
| 9. | Type II vs. no processes | **< 0.001** |
| 10. | Type III vs. type IV | 0.015 |
| 11. | Type III vs. type V | **0.001** |
| 12. | Type III vs. no processes | 0.076 |
| 13. | Type IV vs. type V | 0.184 |
| 14. | Type IV vs. no processes | **< 0.001** |
| 15. | Type V vs. no processes | **< 0.001** |

p value < 0.01 is significant

### *Moderately differentiated squamous cell carcinoma of cervix*

Two moderately differentiated squamous cell carcinoma specimens with nodal metastasis had a large number of CD1a positive LCs. In the epithelium, all five types of LCs were noted and in addition, there were many cells without any cell processes. These cells were rounded, less intensely stained and lacked cytoplasmic processes (Figure 13). In the region where lymphocytic infiltration was seen in the epithelium, large numbers of CD1a positive LCs were noted (Figure 14). The remaining specimens with moderately differentiated squamous cell carcinoma showed a low number of CD1a positive cells. The mean number of LCs per mm length of ectocervical epithelium was 6.82 ± 3.76 (Table 9).

**Table 9: Mean number of CD1a positive LCs per mm length of ectocervical epithelium in poorly and moderately differentiated squamous cell carcinoma of cervix**

| Squamous cell carcinoma of cervix | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Moderately differentiated | 6.82 | 3.76 | 1.64 | 11.48 |
| Poorly differentiated | 4.14 | 1.15 | 2.68 | 5.64 |

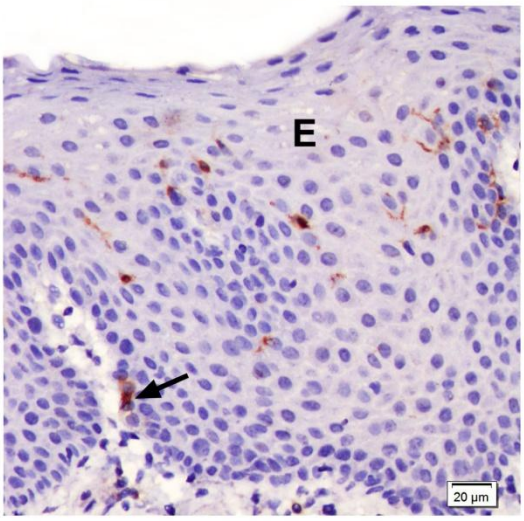



Figure 13: Moderately differentiated squamous cell carcinoma of cervix showing predominantly round, less intensely stained CD1a positive LCs (brown cells) without dendritic processes in the ectocervical epithelium (E). Arrow indicates a CD1a positive LC migrating through the basement membrane.

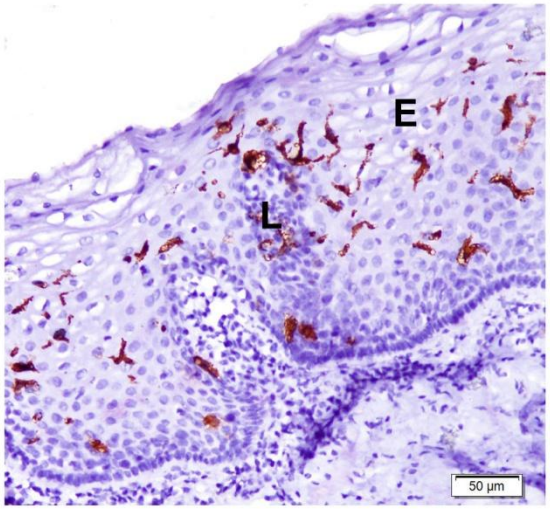



Figure 14: Moderately differentiated squamous cell carcinoma of cervix with nodal metastasis showing increased number of CD1a positive LCs (brown cells) around a lymphocytic infiltration (L) in the ectocervical epithelium (E).

In the lamina propria of moderately differentiated squamous cell carcinoma, a few CD1a positive cells were present. In cases with nodal involvement, there was a dense infiltration of LCs and lymphocytes in the underlying lamina propria (Figure 15). Large numbers of LCs was seen in the lamina propria where there were lymphocytic aggregations (Figure 16). Migration of LCs across the basement membrane was observed. LCs were also seen in close association with blood vessels (Figure 17).

## *Poorly differentiated Squamous cell carcinoma of cervix*

Low numbers of CD1a positive LCs were noted in all cases of poorly differentiated squamous cell carcinoma (Figure 18). Although the number of cells per mm was relatively low, wherever there were infoldings of basement membrane, large numbers of LCs were noted (Figure 19). The mean number of LCs per mm length of ectocervical epithelium was 4.14 ± 1.15 (Table 9).

Though the number of CD1a positive LCs was sparse in the intact epithelium away from the tumour area, dense infiltrations of CD1a positive LCs was noted in the tumour area and its adjacent epithelium (Figure 20). A few LCs were present in the lamina propria (Figure 21). In addition, HEVs with lymphocytes in their lumina were seen in the lamina propria. Close apposition of LCs and the lymphocytes were demonstrated in the lumina of HEVs. CD1a positive LCs were seen in close contact with the walls of HEVs (Figure 22).

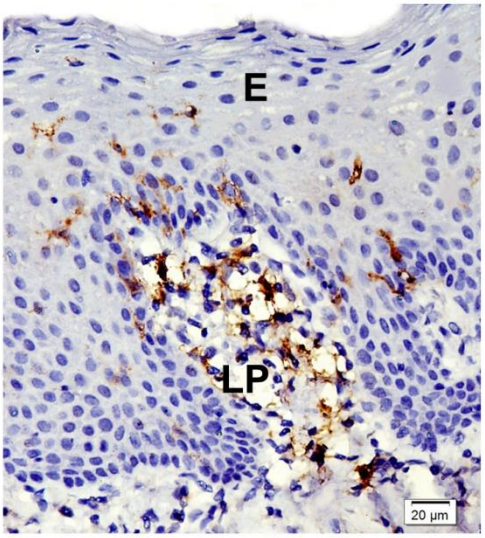



Figure 15: Moderately differentiated squamous cell carcinoma of cervix with nodal metastasis showing increased number of CD1a positive LCs (brown cells) in the lamina propria (LP); E- epithelium.

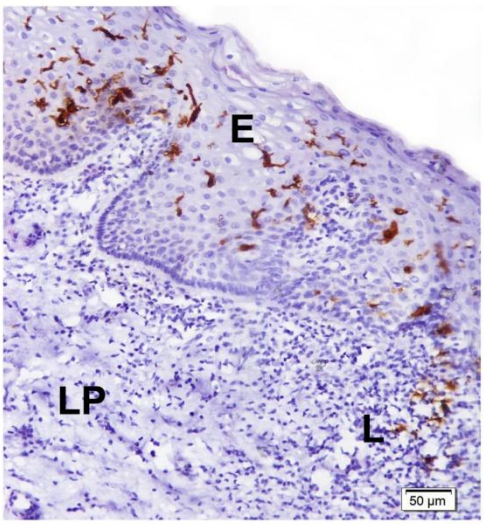



Figure 16: Moderately differentiated squamous cell carcinoma of cervix with CD1a positive LCs (brown cells) in a lymphocytic infiltration (L) present in the lamina propria (LP); E- epithelium.

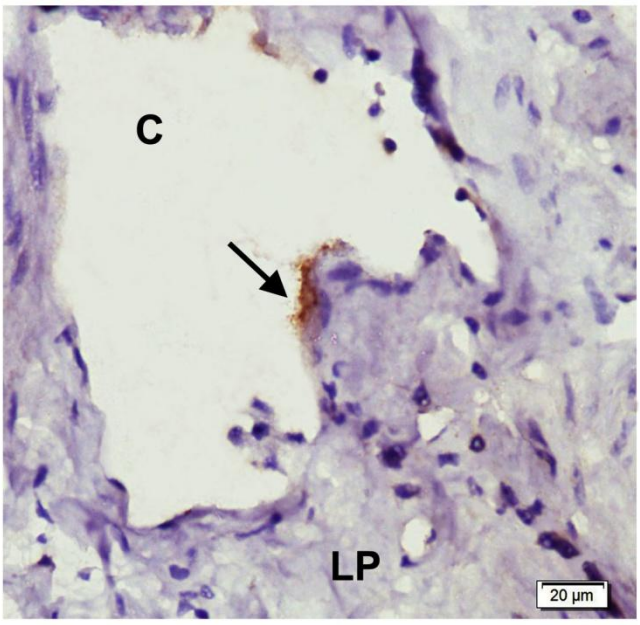



Figure 17: Arrow indicates a CD1a positive LC present close to the capillary (C) wall in the lamina propria (LP) in a case of moderately differentiated squamous cell carcinoma of cervix.

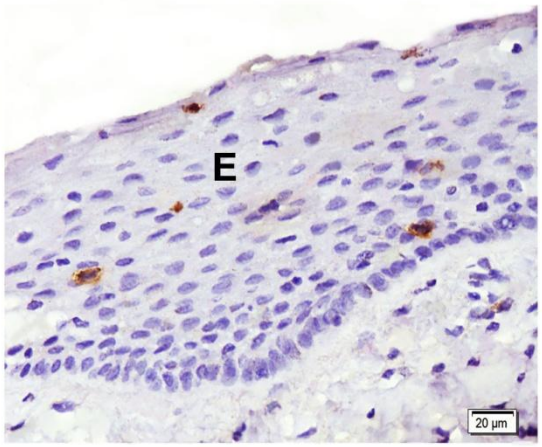



Figure 18: A few rounded CD1a positive LCs (brown cells) with no processes in the ectocervical epithelium (E) in a case of poorly differentiated squamous cell carcinoma of cervix.

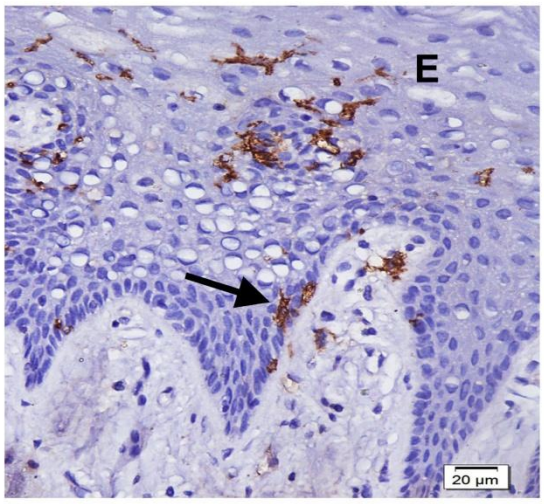



Figure 19: A case of poorly differentiated squamous cell carcinoma of cervix showing many CD1a positive LCs (brown cells) in the ectocervical epithelium (E) overlying a region of basal infolding. Arrow indicates migration of LCs.

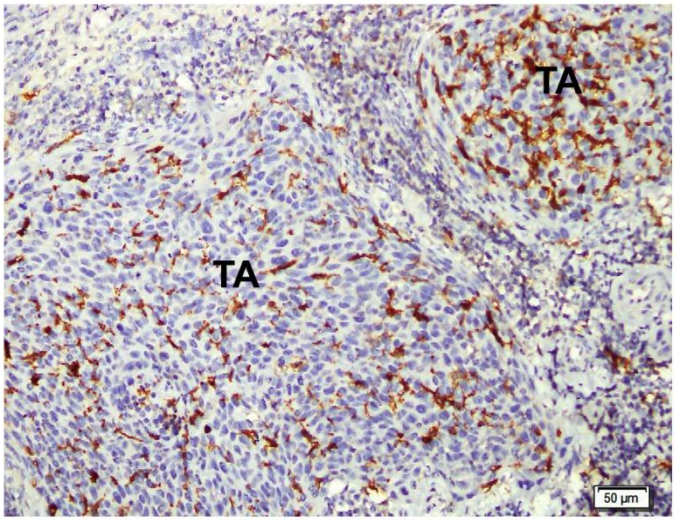



Figure 20: Note dense infiltration of CD1a positive LCs (brown cells) in the tumour area (TA) in a case of poorly differentiated squamous cell carcinoma of cervix.

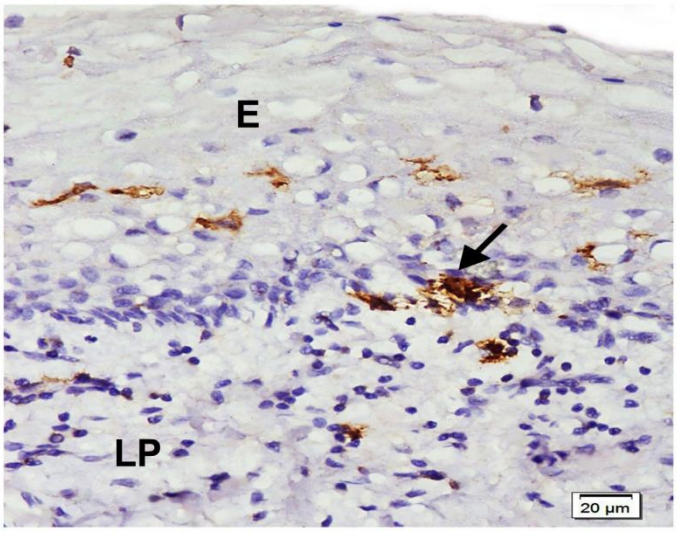



Figure 21: A case of poorly differentiated squamous cell carcinoma of cervix showing the presence of LCs (brown cells) in the lamina propria (LP) and in the ectocervical epithelium (E). Arrow indicates the migration of CD1a positive LC through the basement membrane.

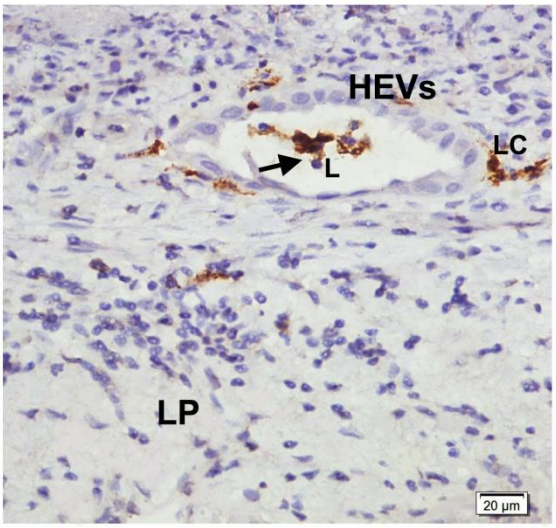



Figure 22: Poorly differentiated squamous cell carcinoma of cervix showing a high endothelial venule (HEV) in the lamina propria (LP). Note the presence of Langerhans cell (LC) surrounding the wall of the HEV. Arrow indicates the close apposition of a CD1a positive LC with lymphocytes (L) in the lumen of the HEV.

## Adenocarcinoma of cervix

The CD1a positive LCs were scattered through all the layers of the stratified squamous epithelium in adenocarcinoma of cervix. Their mean number per mm length of ectocervical epithelium was 3.63 ± 2.09. The diameters of the CD1a positive Langerhans cells ranged from 3.24 to 14.51μm with a mean diameter of 7.56 ± 1.95μm (Table 10). Moderately differentiated adenocarcinoma had type I and type III cells in addition to cells with no processes (Chart 4 and Table 11). There was one specimen of a well differentiated adenocarcinoma which had a few CD1a positive LCs in the epithelium, which were predominantly rounded without processes (Figure 23). Large numbers of LCs were noted in the regions of epithelium with lymphocytic infiltrations (Figure 24). A few LCs were found in the lamina propria.

**Table 10: The number of CD1a positive LCs per mm length of ectocervical epithelium and diameter of cells in the of adenocarcinoma of cervix**

| Adenocarcinoma of cervix | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Number | **3.63** | 2.09 | 1.40 | 5.64 |
| Diameter (μm) | 7.56 | 1.95 | 3.24 | 14.51 |

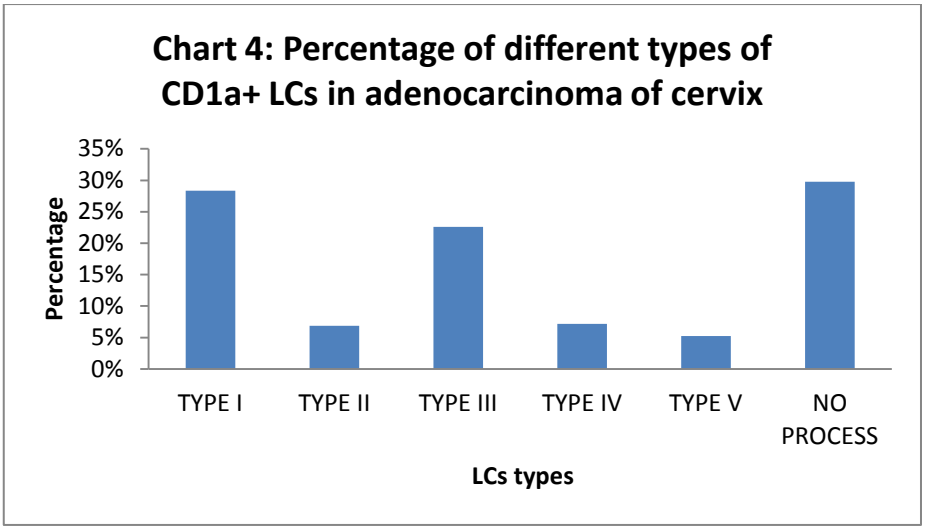



**Table 11: Distribution of different types of CD1a positive LCs per mm length of ectocervical epithelium in adenocarcinoma of cervix and comparison of all types of LCs using the Kruskal Wallis test.**

| Types of LCs | Mean | Standard deviation | Minimum | Maximum | Percentage | p value |
|---|---|---|---|---|---|---|
| Type I | 1.03 | 0.64 | 0.52 | 1.96 | 28.37% | |
| Type II | 0.25 | 0.31 | 0.00 | 0.64 | 6.89% | |
| Type III | 0.82 | 0.66 | 0.24 | 1.52 | 22.59% | |
| Type IV | 0.26 | 0.33 | 0.00 | 0.72 | 7.16% | **0.043** |
| Type V | 0.19 | 0.33 | 0.00 | 0.68 | 5.23% | |
| No processes | 1.08 | 0.51 | 0.60 | 1.72 | 29.75% | |

$p < 0.05$ is significant

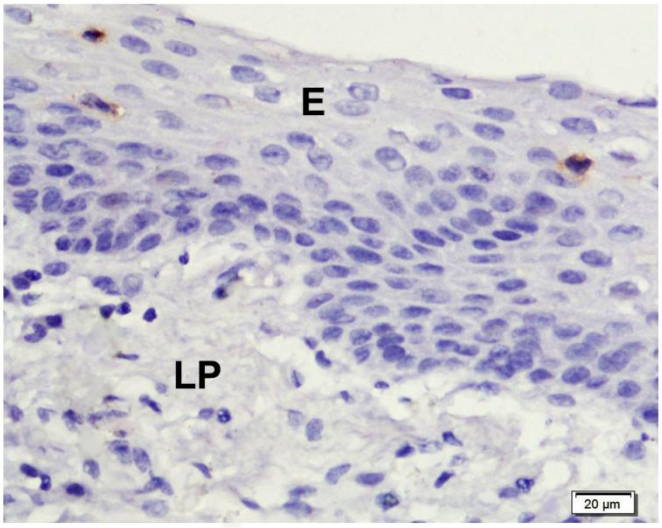



Figure 23: A few rounded CD1a positive LCs (brown cells) without dendritic processes in the ectocervical epithelium (E) in a case of well differentiated adenocarcinoma of cervix; LP- lamina propria.

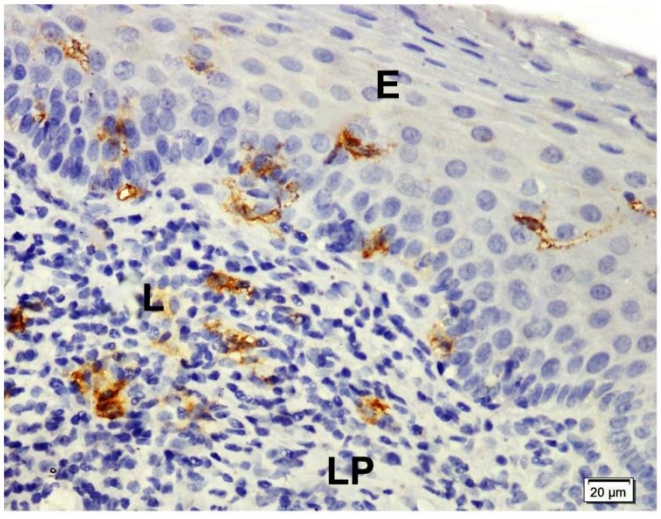



Figure 24: CD1a positive LCs (brown cells) are confined to the basal and suprabasal layers of ectocervical epithelium (E) in a case of endometroid adenocarcinoma. Note the large number of LCs within a lymphocytic infiltrations (L) present in the lamina propria (LP).

## Normal cervix associated with other genital carcinomas

The CD1a positive LCs were seen mainly in the basal and suprabasal layers of the ectocervical epithelium. Their mean number per mm length of ectocervical epithelium was 5.51 ± 0.99. The diameter of the CD1a positive LCs ranged from 3.92 to 13.23μm with a mean diameter of 7.90 ± 1.69μm (Table 12). Most of them were type I cells and cells with no processes (Chart 5 and Table 13). The number of type I cells and cells with no processes was significantly higher than type II, type IV and type V cells ($p < 0.001$) (Table 14). Presence of CD1a positive LCs in the lamina propria was observed in regions of lymphocytic infiltrations (Figure 24). A few LCs were seen around the capillary wall in the lamina propria. In this study, in a case of endometroid adenocarcinoma of endometrium, a little endocervical tissue was obtained along with the specimens of ectocervix. In this, CD1a positive LCs were demonstrated in association with the endocervical glands. These cells had typical dendritic morphology although a few cells without dendritic processes were noted (Figures 25a & 25b).

**Table 12: The number of CD1a positive LCs per mm length and diameter of cells in the ectocervical epithelium in the normal cervix associated with other genital carcinomas**

| Normal cervix associated with other genital carcinomas | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Number | 5.51 | 0.99 | 1.32 | 4.68 |
| Diameter (μm) | 7.90 | 1.69 | 3.92 | 13.23 |

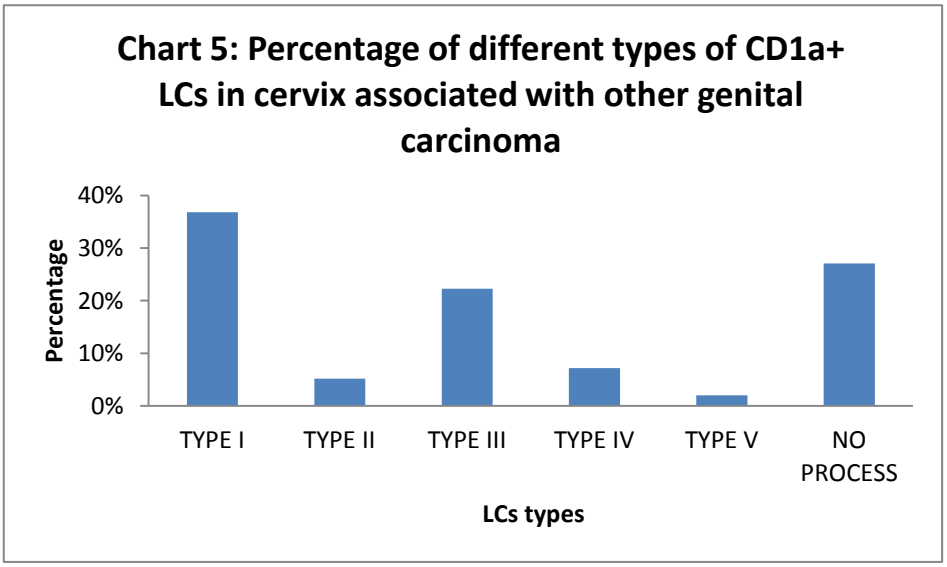


**Table 13: Distribution of different types of CD1a positive LCs per mm length of ectocervical epithelium in the normal cervix associated other genital carcinomas and comparison of all the types of LCs using the Kruskal Wallis test.**

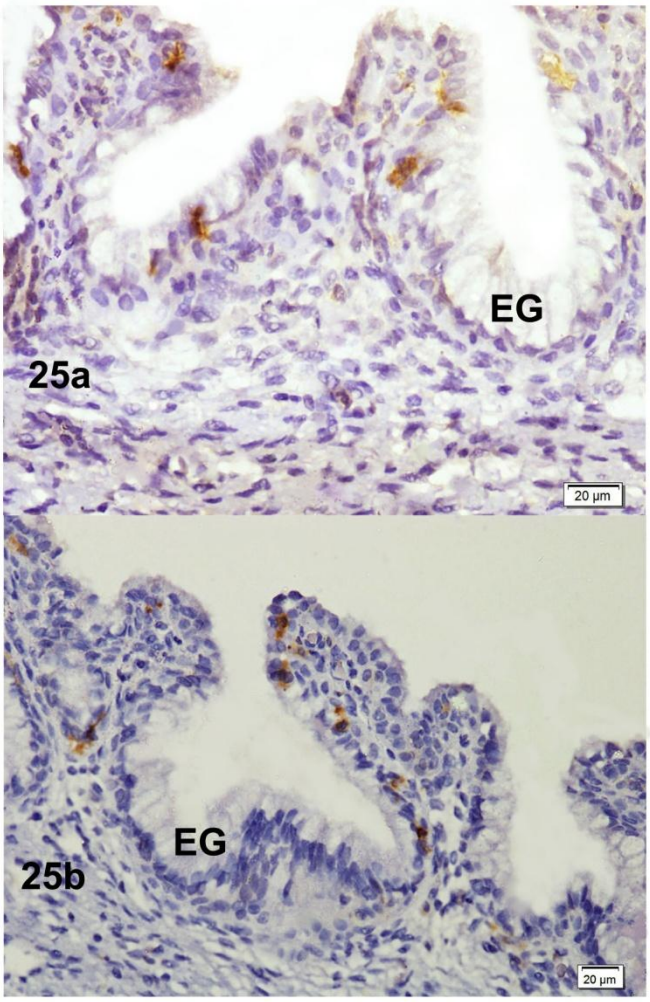
| Types of cells | Mean | Standard deviation | Minimum | Maximum | Percentage | p value |
|---|---|---|---|---|---|---|
| Type I | 1.96 | 1.16 | 0.09 | 4.08 | 36.84% | |
| Type II | 0.28 | 0.24 | 0.01 | 0.64 | 5.18% | |
| Type III | 1.16 | 0.72 | 0.05 | 2.12 | 22.29% | |
| Type IV | 0.40 | 0.41 | 0.01 | 1.04 | 7.15% | **<0.001** |
| Type V | 0.09 | 0.08 | 0.00 | 0.24 | 2.00% | |
| No processes | 1.62 | 0.80 | 0.08 | 2.64 | 27.06% | |

p value < 0.05 is significant

**Table 14: Comparison of the types of LCs within the normal cervix associated with other genital carcinomas using the Mann Whitney U test**

| S.No | Comparison of the types of LCs in the normal cervix associated with other genital carcinomas | p value |
|---|---|---|
| 1. | Type I vs. type II | **0.003** |
| 2. | Type I vs. type III | 0.111 |
| 3. | Type I vs. type IV | **0.005** |
| 4. | Type I vs. type V | **0.001** |
| 5. | Type I vs. no processes | 0.664 |
| 6. | Type II vs. type III | **0.008** |
| 7. | Type II vs. type IV | 0.821 |
| 8. | Type II vs. type V | 0.097 |
| 9. | Type II vs. no processes | **0.003** |
| 10. | Type III vs. type IV | 0.038 |
| 11. | Type III vs. type V | **0.003** |
| 12. | Type III vs. no processes | 0.246 |
| 13. | Type IV vs. type V | 0.060 |
| 14. | Type IV vs. no processes | **0.005** |
| 15. | Type V vs. no processes | **0.001** |

p value < 0.01 is significant




Figures 25a & 25b: Note the presence of many CD1a positive LCs (brown cells) present in the wall of the endocervical glands lined by simple columnar epithelium in a case of moderately differentiated endometroid adenocarcinoma of endometrium.

## I. Comparison of the number of CD1a positive LCs per mm length of the ectocervical epithelium in the categorized five groups.

The mean number of LCs per mm length of ectocervical epithelium was highest in CIN I (10.02 ± 2.01) and lowest in adenocarcinoma of cervix (3.63 ± 2.09)**.** Using the Kruskal Wallis test it was found out that difference in the number of CD1a positive LCs per mm length of ectocervical epithelium between the five categorized groups was statistically significant (p value = 0.004) (Table 15).

The comparison between pairs of groups was done using the Mann Whitney U test. As evident in Table 16, the mean number of LCs was higher in the normal cervix (8 ± 2.76) compared to that of squamous cell carcinoma of cervix (5.36 ± 2.88). There was a statistically significant difference in the number of LCs between these two groups (p value = 0.006). Similarly, the mean number of LCs was higher in the normal cervix (8 ± 2.76) compared to that of adenocarcinoma of cervix (3.63 ± 2.09). This difference was also statistically significant difference in the number of LCs between the normal cervix and adenocarcinoma of cervix (p value **=** 0.003).

**Table 15: Comparison of the number of CD1a positive LCs per mm length of the ectocervical epithelium between the five groups using the Kruskal Wallis test**

| Groups | Number of CD1a positive LCs | | | | p value |
|---|---|---|---|---|---|
| | Mean | Standard deviation | Minimum | Maximum | |
| Normal cervix | 8.00 | 2.76 | 3.84 | 16.04 | |
| CIN I | 10.02 | 2.01 | 8.6 | 11.44 | **0.004** |
| Squamous cell carcinoma of cervix | 5.36 | 2.88 | 1.64 | 11.48 | |
| Adenocarcinoma of cervix | 3.63 | 2.09 | 1.4 | 5.64 | |
| Normal cervix associated with other genital carcinomas | 5.51 | 2.93 | 0.25 | 10.12 | |

$p < 0.05$ is significant

**Table 16: Comparison of the number of CD1a positive LCs per mm length of the ectocervical epithelium between pairs of five groups using the Mann Whitney U test**

| S.No | Comparison between pairs of groups | P value |
|---|---|---|
| 1. | Normal vs. CIN I | 0.214 |
| 2. | Normal vs. squamous cell carcinoma of cervix | **0.006** |
| 3. | Normal vs. adenocarcinoma of cervix | **0.003** |
| 4. | Normal vs. normal cervix associated with other genital carcinomas | 0.058 |
| 5. | CIN I vs. squamous cell carcinoma of cervix | 0.154 |
| 6. | CIN I vs. adenocarcinoma of cervix | 0.133 |
| 7. | CIN I vs. normal cervix associated with other genital carcinomas | 0.089 |
| 8. | Squamous cell carcinoma of cervix vs. adenocarcinoma of cervix | 0.263 |
| 9. | Squamous cell carcinoma of cervix vs. normal cervix associated with other genital carcinomas | 0.657 |
| 10. | Adenocarcinoma of cervix vs. normal cervix associated with other genital carcinomas | 0.368 |
| 11. | Moderately and poorly differentiated squamous cell carcinoma of cervix | 0.126 |

$p < 0.01$ is significant

## II. Comparison of the numbers of different types of CD1a positive LCs per mm length of the ectocervical epithelium in the five categorized groups.

The number of each type of LC per mm length of ectocervical epithelium was compared across the five categorized groups by using the Kruskal Wallis test. There was a statistically significant difference in the number of type I cells among the categorized five groups (p value = 0.002) (Table 17). Similarly, there was a statistically significant difference in the number of type II cells among the categorized five groups (p value = 0.043) (Table 18). However, the difference in the number of other types of LCs namely type III (p= 0.095), type IV (p= 0.147), type V (p=0.987) and no processes cells (p= 0.176) were not statistically significant when compared among the five categorized groups.

The p value obtained by comparing type I and II LCs between pairs of groups using Mann-Whitney U test at 1% level is shown in table 19. The mean number of type I LCs were higher in the normal cervix 2.65 ± 0.99 compared to that of squamous cell carcinoma of cervix 1.57 ± 0.79. There was a statistically significant difference between these two groups (p = 0.002). Similarly, the mean number of type I LCs were higher in the normal cervix 2.65 ± 0.99 compared to that of adenocarcinoma of cervix 1.03 ± 0.64. This difference was also statistically significant (p = 0.003) (Table 19).

**Table 17: Comparison of the type I CD1a positive LCs per mm length of the ectocervical epithelium in the five groups using the Kruskal Wallis test**

| Groups | Number of type I CD1a positive LCs | | | | p value |
|---|---|---|---|---|---|
| | Mean | Standard deviation | Minimum | Maximum | |
| Normal cervix | 2.65 | 0.99 | 1.32 | 4.68 | |
| CIN I | 3.52 | 0.17 | 3.40 | 3.64 | |
| Squamous cell carcinoma of cervix | 1.57 | 0.79 | 0.40 | 3.28 | **0.002** |
| Adenocarcinoma of cervix | 1.03 | 0.64 | 0.52 | 1.96 | |
| Normal cervix associated with other genital carcinomas | 1.96 | 1.16 | 0.09 | 4.08 | |

$p < 0.05$ is significant

**Table 18: Comparison of the type II CD1a positive LCs per mm length of the ectocervical epithelium in the five groups using the Kruskal Wallis test**

| Groups | Number of type II CD1a positive LCs | | | | p value |
|---|---|---|---|---|---|
| | Mean | Standard deviation | Minimum | Maximum | |
| Normal cervix | 0.78 | 0.65 | 0.12 | 2.40 | |
| CIN I | 1.26 | 1.33 | 0.32 | 2.20 | |
| Squamous cell carcinoma of cervix | 0.32 | 0.30 | 0.00 | 0.88 | **0.043** |
| Adenocarcinoma of Cervix | 0.25 | 0.31 | 0.00 | 0.64 | |
| Normal cervix associated with other genital carcinomas | 0.28 | 0.24 | 0.01 | 0.64 | |

$p < 0.05$ is significant

Although the difference between the number of type II LCs in the five groups was statistically significant (p= 0.043), the difference was not statistically significant between any two groups at 1% level (Tables 18 & 19).

**Table 19: Comparison of type I & II CD1a positive LCs per mm length of the ectocervical epithelium between pairs of groups using the Mann Whitney U test**

| S.No | Comparison between pairs of groups | Type I LCs p value | Type II LCs p value |
|---|---|---|---|
| 1. | Normal cervix vs. CIN I | 0.138 | 0.634 |
| 2. | Normal cervix vs. squamous cell carcinoma of cervix | **0.002** | 0.021 |
| 3. | Normal cervix vs. adenocarcinoma of cervix | **0.003** | 0.077 |
| 4. | Normal cervix vs. normal cervix associated with other genital carcinomas | 0.138 | 0.023 |
| 5. | CIN I vs. squamous cell carcinoma of cervix | 0.026 | 0.282 |
| 6. | CIN I vs. adenocarcinoma of cervix | 0.133 | 0.467 |
| 7. | CIN I vs. normal cervix associated with other genital carcinomas | 0.178 | 0.311 |
| 8. | Squamous cell carcinoma of cervix vs. adenocarcinoma of cervix | 0.188 | 0.555 |
| 9. | Squamous cell carcinoma of cervix vs. normal cervix associated with other genital carcinomas | 0.319 | 0.855 |
| 10. | Adenocarcinoma of cervix vs. normal cervix associated with other genital carcinomas | 0.166 | 0.655 |
| 11. | Moderately vs. poorly differentiated squamous cell carcinoma of cervix | 0.160 | 0.946 |

$p < 0.01$ is significant

## III. Comparison of mean diameters of CD1a positive LCs in the five categorized groups

The diameter of hundred CD1a positive LCs was measured per sample. The average diameter of each LC with well defined nucleus was obtained by measuring the maximum diameter and minimum diameters. The range of diameters varied from 3.24 to 17.03μm. The thickness of each section studied was 5μm as it was believed, that the nucleus of a particular cell can be seen only in one section but not in the subsequent section.

The mean diameter of LCs was highest in CIN I (8.75 ± 1.67μm) and lowest in adenocarcinoma of cervix (7.56 ± 1.95μm). The difference in the mean diameters of LCs among the five groups was analysed by ANOVA test. It was found that the mean difference in the diameters of LCs was statistically significant (p value < 0.001) in all five categorized groups (Table 20).

**Table 20: Comparison of the mean diameters of CD1a positive LCs between the five groups by ANOVA test**

| Groups | Mean LC diameter (μm) | Standard deviation | Minimum | Maximum | p value |
|---|---|---|---|---|---|
| Normal cervix | 8.57 | 1.81 | 4.08 | 15.31 | |
| CIN-I | 8.75 | 1.67 | 5.38 | 13.31 | |
| Squamous cell carcinoma of cervix | 8.28 | 2.04 | 3.50 | 17.03 | **<0.001** |
| Adenocarcinoma of cervix | 7.56 | 1.95 | 3.24 | 14.51 | |
| Normal cervix associated with other genital carcinomas | 7.90 | 1.69 | 3.92 | 13.23 | |

p value < 0.05 is significant

The difference in the mean diameters between pairs of groups was done by Scheffe multiple comparison test. As evident in the table 21, the difference in the mean diameters of LCs was statistically significant between the following groups:

The mean diameters of LCs in the

1. normal cervix (8.57 ± 1.81) was larger than that of squamous cell carcinoma of cervix (8.28 ± 2.04) (p = 0.005).

2. normal cervix (8.57 ± 1.81) was larger than that of adenocarcinoma of cervix (7.56 ± 1.95) (p value < 0.001).

3. normal cervix (8.57 ± 1.81) was larger than that of normal cervix associated with other genital carcinomas (7.90 ± 1.69) (p value < 0.001).

4. CIN I (8.75 ± 1.67) was larger than that of adenocarcinoma of cervix (7.56 ± 1.95) (p value < 0.001).

5. CIN I (8.75±1.67) was larger than that of normal cervix associated with other genital carcinomas (7.90±1.69) (p value < 0 .001).

6. squamous cell carcinoma of cervix (8.28 ± 2.04) was larger than that of adenocarcinoma of cervix (7.56 ± 1.95) (p value <0.001).

7. squamous cell carcinoma of cervix (8.28 ± 2.04) was larger than normal cervix associated with other genital carcinoma (7.90 ± 1.69) (p = 0.002).

**Table 21: Comparison of the mean diameters of CD1a positive LCs between pairs of groups using the Scheffe multiple comparison test**

| S.No | Comparison between pairs of groups | 95% confidence interval | | p value |
|---|---|---|---|---|
| | | Lower bound | Upper bound | |
| 1. | Normal cervix vs. CIN I | -0.779 | 0.420 | 0.932 |
| 2. | Normal cervix vs. squamous cell carcinoma of cervix | 0.059 | 0.532 | **0.005** |
| 3. | Normal cervix vs. adenocarcinoma of cervix | 0.685 | 1.346 | **<0.001** |
| 4. | Normal cervix vs. normal cervix with other genital carcinomas | 0.401 | 0.942 | **<0.001** |
| 5. | CIN I vs. squamous cell carcinoma of cervix | -0.128 | 1.078 | 0.208 |
| 6. | CIN I vs. adenocarcinoma of cervix | 0.549 | 1.841 | **<0.001** |
| 7. | CIN I vs. normal cervix with other genital carcinomas | 0.233 | 1.468 | **0.001** |
| 8. | Squamous cell carcinoma of cervix vs. adenocarcinoma of cervix | 0.383 | 1.057 | **<0.001** |
| 9. | Squamous cell carcinoma of cervix vs. normal cervix associated with other genital carcinomas | 0.096 | 0.655 | **0.002** |
| 10. | Adenocarcinoma of cervix vs. normal cervix associated with other genital carcinomas | -0.018 | 0.706 | 0.072 |

p value < 0.01 is significant

# 6. DISCUSSION

Langerhans cells, a type of dendritic cells present in the mucosal layer, are said to be imperative in the recognition and processing of exogenous antigens as a part of immunomonitoring (14). They play an important role in protecting the host by acting as adjuvants and conductors of the immune response against infectious agents and neoplastic changes (55).

Langerhans cells have been demonstrated using various markers like ZIO (26,83,107), ATPase, MHC class II, S100 (108), HLA-DR (15), vimentin (109) and CD1a. In this study to identify the LCs in the cervix, CD1a, a specific and sensitive immuno marker was used (64,65). CD1a is superior to other immune markers which recognize a number of other lymphoreticular cells such as macrophages or melanocytes. Since the only other cell labelled by anti-CD1a antibodies is the immature thymocyte, CD1a is considered as the most specific marker for LCs (13).

Many studies have demonstrated the presence of LCs in normal and pathological cervical tissue (78–80,83,84). Studies have been conducted on LCs in the normal and cancer cervix using various markers like T6, ATPase, MHC class II and S100 and concluded that there are different subpopulations of LCs in cervix (108). Based on the surface markers, different subpopulations of LCs have been reported in other tissues like eyelid (110) and uterine tube (111). Different

subpopulations of LCs reflect functional differences among them (12). In ovarian carcinoma, it was found out that HLA-DR and CD1a positive LCs are more in patients without recurrence when compared to the groups with recurrence group. However S-100 positive LCs are more in the recurrence groups indicating the prognostic value of different subpopulations of LCs in tumours (112). The CD1a positive subpopulation of LCs is functionally distinct and is more important in antitumor immunity than the other subpopulations studied. This is because the CD1a surface antigen is a glycopeptide antigen presenting molecule and is a target in mixed lymphocyte reactions. The ability of LCs to stimulate mixed lymphocyte reactions and to activate T cells in vitro has been blocked by the antibody to CD1a (12).

The morphology and distribution of CD1a positive LCs in the normal and cancerous cervical tissues were considered by immunohistochemistry in this study. In accordance with earlier re[ports (16), in the present study there was regional variation in the distribution of CD1a positive LCs in the epithelium both in the normal and carcinoma specimens. While some areas had a high density of CD1a positive LCs others had no LCs at all.

## Position of LCs in the epithelium

Many previous studies have demonstrated LCs mainly in the basal and suprabasal layers of the stratified squamous epithelium of the normal cervix and vagina (15,75,111), and some in the midzone

(21). One report demonstrated LCs at all levels of the ectocervical epithelium, although preferentially in the intermediate and superficial layers (16). In other tissues which are lined by stratified squamous epithelium like normal oral mucosa, LCs were confined mainly in the suprabasal region. It was also noted in oral squamous cell carcinoma, LCs were distributed more towards the surface (8). In the present study, the CD1a positive LCs were present in the suprabasal and intermediate layers of the stratified squamous epithelium lining the normal ectocervix. It was earlier reported that LCs were distributed throughout the entire thickness of stratified squamous epithelium in CIN except for the Grade I (114). In this study LCs were observed in the suprabasal layers of the ectocervical epithelium in CIN I. However, in the squamous cell carcinoma and adenocarcinoma of the cervix, the cells were distributed in all the layers.

## Number of LCs

There have been contradictory findings regarding the number of LCs in CIN and in cancer cervix. This could be due to the use of different markers representing different subpopulations of LCs in various studies. The distribution of different subpopulation of LCs may vary in normal, CIN and carcinoma.

**Number of LCs in CIN**

Our study demonstrated few LCs in case of dysplasia (82). Others reported a decrease in the number of epithelial CD1a positive LCs in all grades of CIN except CIN I, while using other markers like HLA-DR, HLA-DQ showed increased number of LCs (114). There is also a report of low numbers of LCs in CIN I lesions (79). And another showing low levels of LCs in association with CIN II and III lesions (115). OKT-6 positive LCs (80), T6 positive LCs (108), S-100 positive LCs (108,116,117) and MHC II positive LCs (108,118) were significantly decreased in CIN and HPV infected cervix. Increased number of LCs in CIN when compared with normal also have been reported by different authors by using various markers including S-100, gold chloride and CD1a (14,119–122). In the present study, the mean number of CD1a positive LCs was highest in CIN I when compared with normal and carcinoma cervix. The increased number of LCs in CIN I could be due to host immune response. LCs have been recognised as powerful antigen presenting cells capable of engulfing antigens either by phagocytosis, pinocytosis and endocytosis. They then efficiently present the processed antigens to lymphocytes, inducing a primary immune response.

**Number of LCs in carcinoma cervix**

Using S-100 and OKT6 high density of LCs have been demonstrated among the cells exfoliated from the squamous cell

carcinomas of ectocervix (123). Increased number of LCs in invasive carcinomas have also been noted (54,120,124). The aberrant expression of HLA-DR positive LCs in CIN which is maintained even after the progression of CIN into carcinoma cervix has been demonstrated (125). However other studies have demonstrated reduction in the number of LCs in carcinoma cervix (21,84,126). Reduction in the number of LCs has been reported in other conditions like HPV 16 infection of the epidermis (127), oral squamous cell carcinoma (8) and breast carcinoma (98). In the present study, LCs were more in number in the normal and CIN I whereas squamous cell carcinoma of cervix, adenocarcinoma of cervix and normal cervix associated with other genital carcinomas showed fewer LCs in the epithelium of ectocervix. This is in accordance with authors who reported increased number of LCs in CIN and decreased number of LCs in squamous cell carcinoma of cervix (84). In this present study, the number of CD1a positive LCs was significantly lower in squamous cell carcinoma of the cervix as compared to normal cervix. Similar findings have been reported in the past (126).

The decreased number of LCs in the ectocervical epithelium in carcinoma cervix is due to a suppressed host immune response. Tumours avoid immunosurveillance through various mechanisms including inhibition of recruitment of host DCs and local production of immunosuppressive factors (128). The low number of LCs is responsible for escape of tumour cells from immune recognition and elimination.

Many tumour derived factors including cytokines and inflammatory mediators alter the immune status of an individual especially by targeting LCs differentiation, maturation and also affect lifespan by forming apoptotic DCs and their precursors (129).

## LCs in tumour area

In the present study, ectocervical specimens were collected away from the tumour area but two specimens showed areas infiltrated by tumour. It was noted that although the number of CD1a positive LCs was sparse in the intact epithelium away from the tumour area, there was infiltration of CD1a positive LCs in the tumour area. This could be due to the migration of LCs towards the tumour area. It has been reported earlier that cytokines like IL-10 and VEGF produced by the tumour cells inhibit the maturation of LCs. The immature LCs present in and around the tumour lack the co-stimulatory molecules which are necessary for presenting tumour cell membrane information to the T lymphocytes. This results in ineffective immune response and disease progression (25). On the other hand, several studies have found correlation between the presence and number of LCs within the tumour tissues and clinical prognosis. The tumours infiltrated with large numbers of LCs were associated with better prognosis, longer patient survival and reduced recurrence (130). Higher LCs infiltration in tumour and peritumoral areas was reported to be associated with better prognosis in adenocarcinoma of cervix (131), oesophageal squamous cell carcinomas (93), ovarian cancers (112), tongue squamous cell

carcinomas (12), glottic squamous cell carcinomas (10), gastric carcinomas (68) and thyroid carcinomas (132). However, lack of this correlation in laryngeal squamous cell carcinomas indicates cancer survival is multifactorial as opposed to a single factor like LCs determining the prognosis (13).

## LCs in lymphocyte infiltration

In the present study, large numbers of CD1a positive LCs were noted in regions of lymphocytic infiltration either in the epithelium or lamina propria. This is in accordance with a earlier study demonstrating lymphocytic infiltration with large numbers of LCs both in epithelium and lamina propria of the normal ectocervix (77). Similarly, there was a report of increased number of LCs with evident infiltration of lymphocytes, mainly cytotoxic T cells in patients with laryngeal squamous cell carcinomas (13). While one report showed the presence of S-100 positive LCs predominantly around the lymphocytic aggregates and capillaries in CIN (108), Others demonstrated very few CD1a positive LCs in the lymphoid infiltrates of ductal carcinoma of the breast (97). A study investigating HLA-DR positive LCs and T lymphocytes in and around the tumour nests of squamous cell carcinoma of the oesophagus concluded dense infiltration of LCs and lymphocytes was associated with better prognosis (93).

In addition to the LCs, intraepithelial lymphocytes have been demonstrated in the ectocervical epithelium. In the normal cervix both

CD4 and CD8 subset were demonstrated (133). Recognition by helper lymphocytes of the antigens presented by LCs are important for triggering the immune response (33). However, in cancer cervix, the intraepithelial lymphocytes are predominantly of cytotoxic type (134). Cytotoxic T lymphocyte may control the penetration of antigens by killing epithelial cells bearing exogenous molecules on their surface. Contact between LCs and intraepithelial lymphocytes have been demonstrated (83). The LC-T cell interaction is crucial for T cell activation and differentiation in the neoplastic process (81).

## Types of LCs

The different types of CD1a positive LCs found in the human ectocervix represent different degrees of antigenic stimulation of the cells. Those with the largest number of processes have more surface receptors and display a high antigen-binding activity (16). Figueroa and Caorsi reported type I and type III cells were the most numerous whereas those of Type V were the least frequent in the normal ectocervical epithelium. The present study also showed the cells with single and two processes were more predominant in the normals, those with CIN and carcinoma cervix. Although cells with more processes were scanty, they were relatively more in the adenocarcinoma and squamous cell carcinoma of cervix when compared with normal cervix and CIN I. This is in accordance with the study reporting that most ramified types of LCs were more abundant in the neoplastic exocervix (83). Another study reported that in neoplasia, S-100 positive LCs was

most abundant. The branched type indicates hyperactivity. This hyperactivity may help to overcome the tumour progression (122). In the present study, loss of dendritic processes was demonstrated morphologically by immunostaining with CD1a in CIN I and carcinoma. Cells with no processes were relatively high in CIN I and carcinoma. Loss of dendritic process has also been reported in viral wart infection of cervix (135). The loss of dendritic processes may indicate that immune function of LCs is defective in patients with CIN and cancer. Although the LCs have anti-tumour activity in normal condition, in cancerous lesion they are malfunctioned and polarized into immunosuppressive LCs resulting in tumour progression (87).

## Diameters of CD1a positive LCs

It was found in the present study that the mean diameters of CD1a positive LCs were larger in the normal cervix and CIN I when compared with both squamous cell and adenocarcinomas of the cervix, which could mean LCs in normal and CIN I may be more metabolically active. In cervical carcinoma, cell organelles may be sparse, contributing to the smaller size of the LCs.

## Migration of LCs

In the present study migration of LCs across the basement membrane was observed. GM-CSF plays an important role in migration of LCs. The keratinocytes of the normal cervix induces LC migration by

increased production of GM-CSF but HPV transformed keratinocytes have less capacity to produce the above factor thereby inhibiting the migration of LCs (136). Prevention of LC migration may be a mechanism used by both viruses and tumours to evade activation of host immune responses (137). TNF-α provides an important signal for LC migration and that in the absence of this cytokine, movement of LC from the epidermis to regional lymph nodes is inhibited. Recent evidence indicates that interleukin-1 beta, a cytokine produced in murine epidermis exclusively by LC, may play a role in LC migration across the basement membrane (138).

## LCs in endocervical glands

In this study, CD1a positive LCs were seen in the endocervical glands of endometroid adenocarcinoma of the endometrium which had typical dendritic morphology, although a few cells were noted with loss of dendritic processes Small numbers of vimentin and T6 positive LCs have been demonstrated in the endocervical epithelium of the normal cervix (82). Previous studies have demonstrated other subpopulation of LCs like ZIO positive LCs in the endocervix which were of columnar shape. It was reported that ZIO positive LCs increase in endocervicitis (107). The presence of CD1a positive LCs in the endocervix may indicate the role of this subpopulation of LCs in the local immune response.

## High endothelial venules

In this study HEVs, specialized post capillary venules lined by cuboidal endothelial cells, were noted in the lamina propria of the ectocervix in squamous cell carcinoma. The migration of naïve T and B lymphocytes through HEVs is limited by the LCs surrounding it (105). Previous studies have demonstrated vessels similar to HEVs in the stroma of various carcinomas. The tumour induced HEVs were associated with increased levels of B and T lymphocyte infiltration and good prognosis in breast carcinoma, with inhibition of the tumour metastasis (139). Although the formation of blood vessels and angiogenesis in the tumour area were related to poor prognosis, high levels of HEVs were associated with good prognosis by enhancing the numbers of memory and cytotoxic T lymphocytes infiltrating along with inhibition of the tumour metastasis (139). Chronic inflammatory diseases and cancers can be prevented by enhancing factors involved in maintenance of HEVs (104). The close association of LCs with capillaries and HEV demonstrated in the present study suggests the possible role of LCs in immunosurveillance and the presentation of antigens to lymphocytes.

The sample size determined was 35 each in the normal cervix and squamous cell carcinoma groups. The specimens collected were 22 of normal cervix, and 8 of normal cervix associated with other genital carcinoma. However, only 11 samples were obtained from squamous cell carcinoma of cervix and 4 of adenocarcinoma of cervix. From the data obtained from the collected sample sizes, it was found that there was statistically significant difference in the number of LCs between normal and squamous cell carcinoma and also between normal and adenocarcinoma of cervix and in retrospect this sample size was found adequate to be representative.

In summary, in this study the distribution and morphology of CD1a positive LCs were studied in normal, CIN I, carcinoma of cervix and normal cervix associated with other genital carcinomas. Cancer of the cervix is unique among other cancers because of its preventable nature by screening and removal of precancerous lesions. Dendritic cell-based vaccines have been developed to treat cervical dysplasia and HPV related diseases (140). High accessibility of preventive programs like screening and vaccination is mandatory to overcome carcinoma cervix. The data provided in this study may shed new insights to understand the role of CD1a positive LCs in carcinoma cervix.

# 7. CONCLUSIONS

1. All the cervical specimens showed the presence of CD1a positive LCs.
2. Regional variation was noted in the distribution of LCs.
3. In the normal ectocervix, LCs were predominantly present in the suprabasal and intermediate layers of stratified squamous epithelium.
4. In CIN I LCs were predominantly present in the suprabasal layer of the ectocervical epithelium.
5. In case of carcinoma of cervix, LCs were seen either in the entire thickness, in the suprabasal or mid-epithelial layers, or infrequently, in the superficial layers of the ectocervical epithelium.
6. In addition to the five types of LCs classified by Figueroa and Caorsi (26), LCs with no processes were also noted in all the specimens.
7. While in the normal cervix, CIN I and normal cervix associated with other genital carcinoma LCs predominantly had a single process (Type I), cells with no processes were predominant in the squamous cell carcinoma and adenocarcinoma groups.
8. LC migration through the basement membrane was noted in all the five categorized groups.
9. A large number of LCs was noted associated with lymphocytic infiltrations in both epithelium and lamina propria.

10. LCs were found in close association with the capillaries and HEVs in the lamina propria of cases with squamous cell carcinoma of the cervix.
11. The mean number of CD1a positive LCs per mm length of ectocervical epithelium was highest in CIN I and in normal cervix when compared with carcinoma of cervix and normal cervix associated with other genital carcinomas.
12. The number of CD1a positive LCs was significantly lower in squamous cell and adenocarcinoma of cervix as compared to normal cervix.
13. The number of type I CD1a positive LCs was significantly higher in normal cervix as compared to squamous cell carcinoma of cervix.
14. The number of type I CD1a positive LCs was significantly higher in normal cervix as compared to adenocarcinoma of cervix.
15. Although the number of LCs was sparse in the intact epithelium away from tumour areas, dense infiltrations of LCs were noted in the tumour areas in poorly differentiated squamous cell carcinoma of cervix.
16. The diameters of CD1a positive LCs were significantly higher in normal cervix as compared to squamous cell and adenocarcinoma of cervix and normal cervix associated with other genital carcinomas.

17. The diameters of CD1a positive LCs were significantly greater in CIN I as compared to adenocarcinoma of cervix and normal cervix associated with other genital carcinomas.
18. The diameters of CD1a positive LCs were significantly higher in squamous cell carcinoma of cervix as compared to adenocarcinoma of cervix and normal cervix associated with other genital carcinomas.

# 8. FUTURE SCOPE OF THIS STUDY

1. The distribution and morphology of CD1a positive LCs in CIN of different grades should be studied on more number of samples.
2. Follow up studies of patients with carcinoma cervix included in this study can be done to see the relationship between the number of LCs and prognosis.
3. Number of CD1a positive LCs in different tumour stages of squamous cell carcinoma of cervix with or without lymph node involvement can be studied.
4. Different subpopulation of LCs in carcinoma cervix can be studied using various markers like S-100 and ZIO.
5. Number of LCs in the peritumoural and tumoural areas can be estimated.

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# 10. ANNEXURES

1. IRB approval form

2. Patient information sheet

3. Informed consent form

4. Abbreviations

# INSTITUTIONAL REVIEW BOARD (IRB)
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Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project NEW PROPOSAL:**
The Morphology and Distribution of Dendritic cells in Carcinoma Cervix.
Dr. E. Mohana Priya, Kemp. No. 20880, PG Demonstrator, Anatomy,
Dr. J. Suganthy, Employment no: 30085, Anatomy, Dr. Anitha E Dorairaj, Obstetrics and Gynaecology, Dr. Ramani Kumar, Employment no: 10715, Pathology, Dr. Visalakshi Jeyaseelan, Employment no: 31093, Biostatistics.

Ref: IRB Min. No. 8280 dated 16.04.2013

Dear Dr. E. Mohana Priya,

The Institutional Review Board (Blue, Research and Ethics Committee) of the ChristianMedicalCollege, Vellore, reviewed and discussed your project entitled "The Morphology and Distribution of Dendritic cells in Carcinoma Cervix." on April 16, 2013.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Informed Consent Form and Information Sheet (English and Tamil)
3. Cvs of Drs. E. Mohana Priya, J. Suganthy, Anitha E Dorairaj, Visalakshi Jeyaseelan, Ramani Kumar
4. A CD containing documents 1 – 3


TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

# INSTITUTIONAL REVIEW BOARD (IRB)
# CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

**Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)**
Director, Christian Counselling Centre
Chairperson, Ethics Committee

**Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho**
Chairperson, Research Committee & Principal

**Dr. Nihal Thomas**
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)


The following Institutional Review Board (Research & Ethics Committee) members were present at the meeting held on April 16, 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

| Name | Qualification | Designation | Other Affiliations |
|---|---|---|---|
| Dr. Benjamin Perakath | MBBS, MS, FRCS | Professor, Surgery (Colorectal), CMC. | Internal, Clinician |
| Dr. Balamugesh | MBBS, MD(Int Med), DM, FCCP (USA) | Professor, Dept. of Pulmonary Medicine, CMC. | Internal, Clinician |
| Dr. Anup Ramachandran | PhD | The Wellcome Trust Research Laboratory Gastrointestinal Sciences | Internal |
| Dr. Ellen Ebenezer Benjamin | M.Sc | Maternity Nursing, CMC | Internal, Nurse |
| Dr. Denny Fleming | BSc (Hons), PhD | Honorary Professor, Clinical Pharmacology, CMC. | Internal, Pharmacologist |
| Dr. Srinivas Babu | M.Sc, Ph.D. | Sr. Scientist, Neurological Sciences, CMC. | Internal |
| Dr. Mathew Joseph | MBBS, MCH | Professor, Neurosurgery, CMC | Internal, Clinician |
| Mrs. Pattabiraman | BSc, DSSA | Social Worker, Vellore | External, Lay Person |
| Mr. Sampath | BSc, BL | Advocate | External, Legal Expert |
| Mr. Joseph Devaraj | BSc, BD | Chaplain, CMC | Internal, Social Scientist |
| Dr. B. J. Prashantham (Chairperson), IRB Blue - Internal | MA (Counseling), MA (Theology), Dr Min(Clinica | Chairperson(IRB)& Director, Christian Counselling Centre | External, Scientist |


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Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)


| | | | |
|---|---|---|---|
| Dr. Jayaprakash Muliyil | BSC, MBBS, MD, MPH, DrPH(Epid), DMHC | Retired Professor, Vellore | External, Scientist |
| Mrs. Selva Titus Chacko | MSc | Professor, Medical Surgical Nursing, CMC | Internal, Nurse |
| Dr. Priya Abraham | MBBS, MD, PhD | Professor, Virology, CMC | Internal, Clinician |
| Dr Ashok Chacko | MD, DM, FRCP, FRCPG, FIMSA, FAMS | Director, Institute of Gastroenterology and Liver Disease, Madras Medical Mission, Chennai | External, Clinician |
| Dr. Bobby John | MBBS, MD, DM, PHD, MAMS | Cardiology, CMC | Internal, Clinician |
| Dr. Nihal Thomas | MD MNAMS DNB(Endo) FRACP(Endo) FRCP(Edin) | Secretary IRB (EC)& Dy. Chairperson (IRB), Professor of Endocrinology & Addl. Vice Principal (Research), CMC. | Internal, Clinician |

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.


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*A sum of Rs. 80,000/- (Rupees Eighty Thousand only will be granted for 2 years.* A sum of Rs 40,000/- will be sanctioned for 12 months after receipt of the revised proposal, subsequent installment of 40,000/- each will be released at the end of the first year following the receipt of the progress report.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. J. Suganthy, Department of Anatomy, CMC


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**Christian Medical College, Vellore.**

**Department of Anatomy**

**An observational study on morphometrical analysis of antigen presenting CD1a positive Langerhans cells in human cervical tissues by immunohistochemistry method.**

**Patient Information sheet**

You are kindly requested to participate in this study. As a part of this study after your surgery under Obstetrics and Gynaecology department, a bit of cervical tissue from the removed specimen will be collected. Your hospital chart will be referred for information regarding history, clinical examination and the biopsy report that has been done on you. This information will be utilized to analyze the association between the disease condition and the number of CD1a positive langerhans cells. Participation in this study will not change the quality of the treatment provided by the consulting doctors.

There is no potential harm or complication that can happen to you by participating in this study. All details including personal data will be kept highly confidential.

We hope to include about 60 people from this hospital in this study in the next 2 years. Participation in this study is purely voluntary, and that refusal to participate will not involve any penalty or loss of benefits to which you are otherwise entitled.

In case of doubts/ questions, please contact:

1. Dr. E. Mohana Priya,
   Department of Anatomy,
   Christian Medical College,
   Vellore- 632002. Mobile no: 9865341757

2. Dr. Anitha E Dorairaj,
   Department of Obstetrics and Gynaecology, Unit 1,
   Christian Medical College,
   Vellore- 632004.

| |
|---|
| Study ID No:<br><br>Hospital No: |

**Christian Medical College, Vellore.**

**Department of Anatomy**

**An observational study on morphometrical analysis of antigen presenting CD1a positive Langerhans cells in human cervical tissues by immunohistochemistry method**

**Informed consent form**

(i) I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

(iii) I understand and agree that the Sponsor of this study, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my hospital records both in respect of this study and any further research that may be conducted in relation to it, even if I withdraw from the same. However, I understand that my identity will not be revealed in any information released to third parties or when published.

To this, I agree to take part in the above study.

1. Signature (or Thumb impression) of the patient:
   Date:
   Patient's Name:

2. Signature of the witness:
   Date:
   Name of the witness:

3. Signature of the Investigator
   Date:
   Study investigator's Name:

## ABBREVIATIONS

| | |
|---|---|
| DCs | Dendritic cells |
| MHC | Major histocompatibility complex |
| LCs | Langerhans cells |
| HPV | Human papilloma virus |
| IL-10 | Interleukin – 10 |
| VEGF | Vascular endothelial growth factor |
| CD | Cluster differentiation |
| TGF- β1 | Transforming growth factor beta 1 |
| CIN | Cervical intraepithelial neoplasia |
| SCC | Squamous cell carcinoma |
| MDCs | Myeloid dendritic cells |
| PDCs | Plasmacytoid dendritic cells |
| GM-CSF | Granulocyte macrophage colony stimulating factor |
| INF- α | Interferon alpha |
| HLA-DR | Human leukocyte antigen |
| TRAIL | Tumour necrosis factor related apoptosis inducing ligand |
| OED | Oral epithelial dysplasia |
| HEVs | High endothelial venules |
| PLL | Poly- L- lysine |
| HRP | Horseradish peroxidase |
| PBS | Phosphate buffered saline |
| DAB | Diaminobenzidine |
| EDTA | Ethylene diamine tetra acetic acid |
| TBS | Tris buffered saline |