STUDY OF EXPRESSION OF CYCLOOXYGENASE-2 IN CEVICAL INTRA EPITHELIAL NEOPLASIA AND CERVICAL CANCER

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"A STUDY OF EXPRESSION OF CYCLOOXYGENASE-2 IN CERVICAL INTRA EPITHELIAL NEOPLASIA AND CERVICAL CANCER."

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IN

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LIST OF ABBREVIATIONS

ABBREVIATION	PARAMETER
COX	Cyclooxygenase
CIN	Cervical Intraepithelial Neoplasia
SCC	Squamous Cell Carcinoma
LVI	Lymphovascular Invasion
ACC	Adenocarcinoma
ASC	Adenosquamous Carcinoma
LSIL	Low-grade Intraepithelial Lesion
HSIL	High-grade Intraepithelial Lesion
EGFR	Epidermal Growth Factor Receptor
VEGF	Vascular Endothelial Growth Factor
PG	Prostaglandin
HPV	Human Papilloma Virus
NOS	No Otherwise Specified
DNA	Deoxyribonucleic acid
LCR	Long Control Chain
HTLV	Human T-Cell Leukaemia Virus
EBV	Epstein-Barr Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IHC	ImmunoHistoChemistry
FFPE	Formalin-Fixed Paraffin-Embedded
CEA	Carcinoembryonic Antigen

HRP	Horseradish Peroxidase
ß-HCG	ß-Human Chorionic Gonadotropin
TNF – α	Tumor Necrosis Factor – α
IL-1	Interleukin-1
IFN	Interferon-Gamma

ABSTRACT

INTRODUCTION

The second most common cause of mortality for women worldwide and in developing nations is cervical cancer. Cyclooxygenase (COX)-1 and 2 are two isoenzymes that have different morphological and biological properties. Growth factors, tumor-promoting agents, oncogenes, and carcinogens do activate COX-2, which promotes the proliferation, growth, and spread of tumors. COX-2 is overexpressed in malignant cells and transformed cells. So we will evaluate the expression pattern of COX-2 in cervical intraepithelial neoplasia (CIN), and in different types of cervical cancer, and its correlation with parametrial and lymphovascular invasion and tumor differentiation for the patient's therapeutic benefit.

OBJECTIVES

To analyze the differential expression pattern of COX-2 in CIN and different types of cervical cancer and its correlation with tumor differentiation, parametrial, and lymphovascular invasion.

METHODS

This retrospective and prospective study of 62 samples in histologically diagnosed cases of in-situ and malignant lesions of the cervix was conducted in the department of pathology, BLDE's Shri B.M.Patil Medical College, Vijayapura. Paraffin blocks of histologically diagnosed cases of carcinoma of the cervix (in-situ, squamous cell carcinoma, and adenocarcinoma) were retrieved from the Histopathology Section. The tissue was processed routinely. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis and the other sections were subjected to COX-2 immunohistochemical staining. Cases of colon carcinoma were taken as positive controls. Cytoplasmic and membrane staining of tumor cells were considered as positive staining and the grading was done.

RESULTS

Out of the 62 patients, 40 cases (64.5%) showed positive expression of COX-2 in SCC, when compared with in-situ CIN and ACC. The results were statistically significant with p value 0.003.

CONCLUSION

COX-2 expression is directly proportional to the level of grading of the tumor. The higher the grading higher is the expression of COX-2. Selective COX-2 inhibitors increase the efficacy of chemotherapy or radiotherapy.

KEYWORDS

COX-2, High grade, Overexpression.

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"A STUDY OF EXPRESSION OF CYCLOOXYGENASE-2 IN CERVICAL INTRA EPITHELIAL NEOPLASIA AND CERVICAL CANCER."

INTRODUCTION

The second most common cause of death in women worldwide is cervical cancer, and it is a major cause of death in the developing world. In developed countries such as the United States in the year 2000, it was reported to be 12,800 new cases of cervical cancer and 4,600 death. It is the most prevalent issue in developing nations, and worldwide, nearly 500,000 new cases of cervical cancer are discovered each year[1].

Cyclooxygenase (COX)-1 and 2 are two isoenzymes, in the presence of free arachidonic acid synthesize prostaglandin which is an active lipid compound. Along with the inflammatory activity, prostaglandins are also involved in the growth of the tumor and even in carcinogenesis.[2]

COX-1 and COX-2 have different properties, morphologically and biologically. COX-1 is a housekeeping gene, whereas COX-2 is an immediate-early response gene. COX-2 is induced by growth factors, tumor promoters, oncogenes, and also carcinogens.[1] By mediating pathologic processes which affect mitogenesis, cellular adhesions, and even immune surveillance, COX-2 promotes the proliferation, growth, and spread of the tumor. In transformed cells and malignant tissues, COX-2 is overexpressed. Few studies show inhibition of apoptosis and tumor angiogenesis stimulation by COX-2. Selective COX-2 inhibitors increase the efficacy of chemotherapy or radiotherapy in the treatment of human cancer.

Infection with human papillomavirus is the main cause of carcinoma of the cervix. Viral oncogenic proteins (E5, E6, and E7) increase the expression of COX-2 by activating the COX-2/prostaglandin E2 pathway, and it can be a significant prognostic marker for uterine cervical carcinomas.[1,2,3]

During early stages of the cell replication or differentiation, "COX-2" is present in the cells. In high-grade SCC of the esophagus, adenomatous and metaplastic lesions of the stomach, pre-neoplastic lesions of the lung, pre-invasive neoplasias of the bladder, pancreas, and breast expression of COX-2 is increased.[4] There are few studies that demonstrated the expression of COX-2 in cervical cancer, so in this study, we will evaluate the expression pattern of COX-2 in cervical intraepithelial neoplasia(CIN), and in different types of cervical cancer and its correlation with parametrial and lymphovascular invasion and tumor differentiation.

OBJECTIVES OF THE STUDY

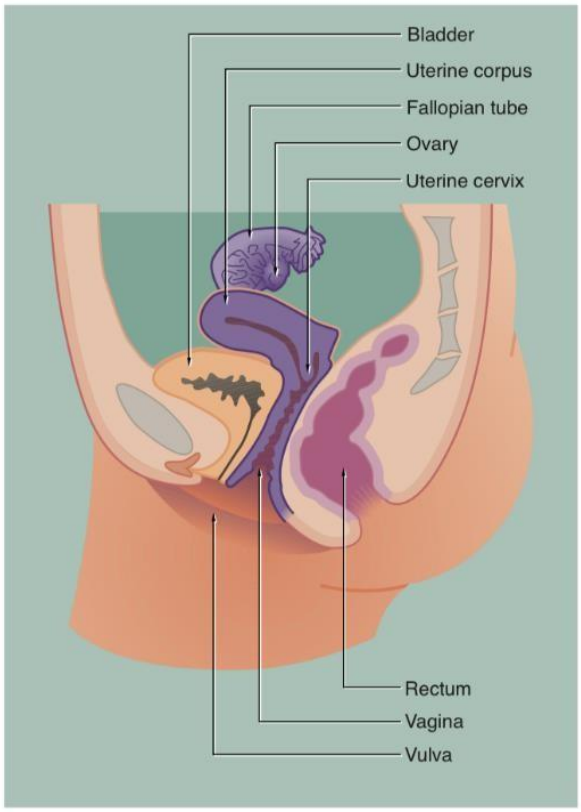
To analyze the differential expression pattern of "COX-2" in CIN and different types of cervical cancer and its correlation with tumor differentiation, parametrial, and lymphovascular invasion.

REVIEW OF LITERATURE

Normal anatomy

Cervix is the lowest region of the uterus which joins this organ to the vagina.
The portio vaginalis, which protrudes into the vagina, and the area above the vaginal vault ma ke up this structure (supravaginal portion). The term "ectocervix" refers to the surface of the portio vaginalis that faces outward, and the term "endocervix" refers to the area that faces the endocervical canal. The term "external os" refers to the endocervical canal's opening onto the ectocervix, whereas "internal os" refers to the endocervical canal's vague upper limit (Figure-1).[5]

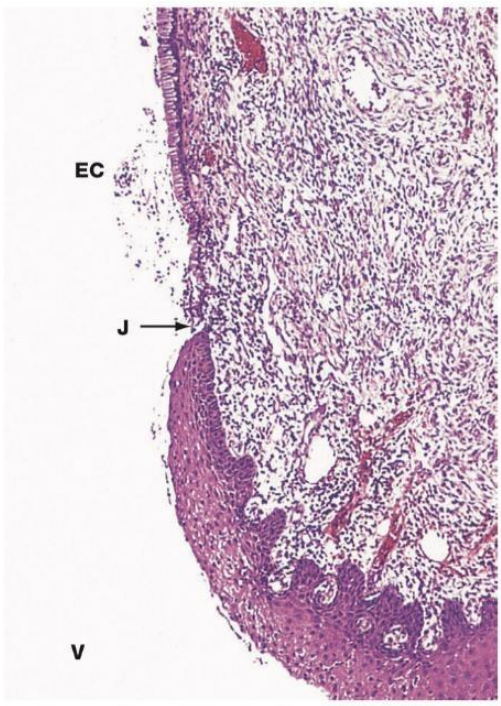
FIGURE-1: Sagittal View Of Female Reproductive System (Courtesy: Wheater's functional histology; 6th edition)



Histology of cervix:

The cervix protrudes into the upper part of the vagina and contains the endocervical canal, which connects the uterine cavity with the vagina. The endocervical canal (EC) is lined with a single layer of tall, columnar, mucus-secreting epithelial cells. Like the vagina and vulva, the cervix is lined with a thick stratified squamous epithelium. Cervical cells often have a distinct cytoplasm due to their high glycogen content. The transition between the ectocervical epithelium and the epithelium of endocervic is very abrupt and is usually located at the ostium, the point where the cervix meets the vagina. The main part of the cervix is made up of tough collagenous tissue and smooth muscle. In the squamous columnar junction, the cervical stroma is often infiltrated with leukocytes and forms part of the defense against microbial invasion. (Fig 2).[5]

FIGURE-2: Cervix Normal Histology (H&E, Whole Mount,4X) EC-Endocervix, V- Ectocervix, J-Transformation zone. (Courtesy:Wheater's functional histology;6th edition)



WHO CLASSIFICATION OF TUMOURS OF THE UTERINE CERVIX-2020[6]

Squamous epithelial tumors

- Squamous metaplasia
- Atrophy
- Condyloma acuminatum
- Low-grade squamous intraepithelial lesion :
  - Cervical intraepithelial neoplasia, grade 1.
- High-grade squamous intraepithelial lesion :
  - Cervical intraepithelial neoplasia, grade 2,
  - Cervical intraepithelial neoplasia, grade 3.
- Squamous cell carcinoma, HPV associated
- Squamous cell carcinoma, HPV independent
- Squamous cell carcinoma, NOS.

Glandular tumors and precursors

- Endocervical polyp
- Müllerian papilloma
- Nabothian cyst
- Tunnel clusters
- Microglandular hyperplasia
- Lobular endocervical glandular hyperplasia
- Diffuse laminar endocervical hyperplasia

- Mesonephric remnants and hyperplasia
- Arias Stella reaction of the uterine cervix
- Endocervicosis
- Tuboendometrioid metaplasia
- Ectopic prostate tissue
- Adenocarcinoma in situ, NOS
- Adenocarcinoma in situ, HPV associated
- Adenocarcinoma in situ, HPV independent
- Adenocarcinoma, NOS
- Adenocarcinoma, HPV associated
- Adenocarcinoma, HPV independent, gastric type.
- Adenocarcinoma, HPV independent, clear cell type
- Adenocarcinoma, HPV independent, mesonephric type
- Adenocarcinoma, HPV independent, NOS
- Endometrioid Adenocarcinoma, NOS
- Carcinosarcoma NOS
- Adenosquamous Carcinoma
- Mucoepidermoid carcinoma
- Adenoid basal carcinoma
- Carcinoma, undifferentiated, NOS

- **Mixed epithelial and mesenchymal tumors**
  - Adenomyoma, NOS
    - Mesonephric type adenomyoma
    - Endocervical type adenomyoma
  - Adenosarcoma
- **Germ cell tumors**
  - Germ cell tumor, NOS
    - Mature teratoma, NOS
    - Dermoid cyst, NOS
    - Endodermal sinus tumor
    - Yolk sac tumor, NOS
    - Choriocarcinoma, NOS

"HUMAN PAPILLOMA VIRUS (HPV) AND THE LOWER FEMALE GENITAL TRACT"

There are about 70 genetically different varieties of HPV, a family of DNA viruses, with types 6 and 11 being the most prevalent, carrying high risk of developing into squamous cell carcinoma of the cervix. Other types (e.g., types 1, 2, 4, and 7) cause benign squamous papillomas (warts) in humans. Currently, HPVs are separated into major categories based on how much they are linked to invasive squamous cell carcinoma and CIN. The most prevalent types worldwide, accounting for 85% of cases, were determined to be HPV types 16, 18, 31, 33, and 45 in a recent large-scale analysis of the distribution of HPV types in invasive cervical cancer.

Electron microscopy can identify it (as intranuclear crystalline and occasionally filamentous inclusions), but immunohistochemical or molecular virologic examination with in situ or Southern blot hybridization is required for its precise identification. Currently, the latter test is recognised as the "gold standard" for HPV detection. HPV infection is transmitted sexually, preferring the metaplastic squamous epithelium. It may become active and release

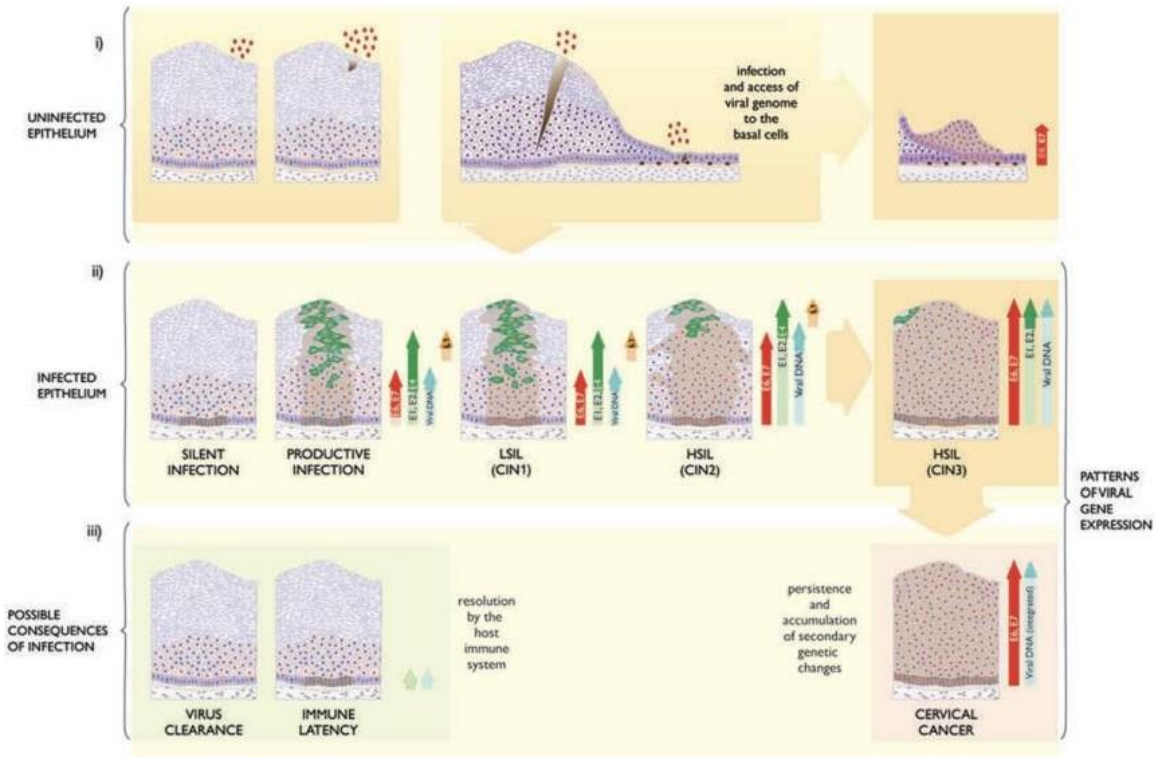
an infectious virus in the terminally differentiated squamous epithelium, or it may remain dormant for extended periods of time.

Pathogenesis of HPV infection:

Infection requires entry of HPV virions into mitotically active epithelial cells of the basal layer, and possibly microwounding in the multilayered epithelium. In the columnar cell layer, infection is thought to be facilitated by the proximity of target cells to the epithelial surface. This gives the virus access to cell types that cannot support a full productive life cycle. The importance of infecting different cell types has not yet been properly assessed.
Expression of the viral genome may be suppressed (for example, by methylation of the genome) and the viral genome remains in the basal layer, resulting in a 'silent' infection with no apparent disease. Alternatively, infection may result in an ordered pattern of viral gene expression, resulting in virus synthesis and release from the upper epithelial layer, leading to CIN.

In most cases, HPV infections tend to resolve as a result of a cell-mediated immune response. This may lead to viral clearance or viral latency and persistence of viral episomes in the basal epithelial layer without completing the life cycle. Viral gene expression patterns during latency are not well characterized. The persistent deregulated gene expression that occurs after CIN3 and viral genome integration can lead to the accumulation of secondary genetic alterations and cancer development in infected host cells. This is facilitated by overexpression of the high-risk proteins E6 and E7. In cervical cancer, the viral genome often integrates, resulting in loss of expression of full-length E1, E2, E4, and E5 and L1 and L2 capsid proteins, and deregulated expression of E6 and E7. Due to their interactions with P53 and RB gene product respectively, the HPV proteins E6 and E7 are believed to be crucial players in the pathogenesis of cervical cancer.

FIGURE 3- Pathogenesis of HPV. (Courtesy: WHO Classification of tumors of female reproductive organs, 4th edition)

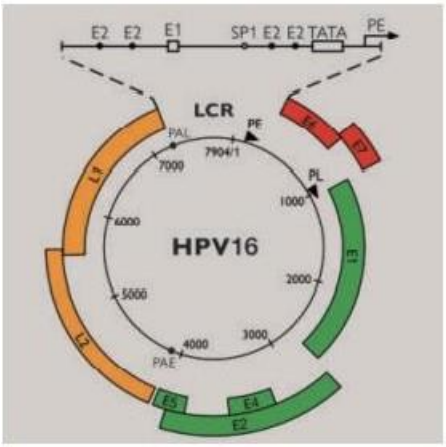


i. Viral particles are present on uninfected epithelial surfaces (eg, due to recent infection). or latent or silent infection.

ii. Red nuclei in the above figure are used to denote the cells in cycle. Green is used to represent the cells expressing E4, whereas yellow is used to represent the cells expressing L1. All the cells that contain the viral genome (differentiated and undifferentiated) are identified by brown shading.

iii. Resolution of HPV infection as a result of cell mediated immune response. Red nuclei are used to denote the cells in cycle. The cells harbouring viral episomes are indicated by brown shading in the immune latency state (Fig 3).

FIGURE 4- The Genomic Composition Of HPV-16. (Courtesy: WHO Classification of tumors of female reproductive organs, 4th edition)



The long control region (LCR) and eight genes that regulate various phases of the viral life cycle make up HPV-16's genome. Due to mRNA splicing, these genes produce a greater variety of gene products. The viral E1 and E2 proteins that regulate viral replication and gene expression, as well as cellular transcription factors, have binding sites in the LCR (Fig 4).[6]

"PRECURSOR LESIONS OF SQUAMOUS CELL CARCINOMA OF CERVIX"

CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

Terminology for cervical intraepithelial lesions made of squamous epithelium and presumed to be invasive carcinoma precursors has changed throughout time and is still evolving. The hypothesis that there are morphologically distinguishable precursor lesions of cancer has been based on this premise, making it both historically and practically significant.

HISTOPATHOLOGICAL FEATURES:

CIN1/LSIL:

It is characterised by basaloid morphology, low nuclear-to-cytoplasmic ratio cells with enlarged, hyperchromatic nuclei in the superficial mucosa, full-thickness atypia, moderate to abundant cytoplasm in cells within the upper two thirds of the epithelium, and significant mitotic activity should be restricted to the lower epithelial third. Additionally, middle and surface cell koilocytic atypia are observed. In 90% of cases, there is also evidence of binucleation.[6,7]

CIN2/HSIL:

In this full-thickness atypia, basaloid cells make up the majority of the cells, and mitotic activity can be seen in the upper two thirds of the epithelium. Disorderly arranged cells exhibit crowding and loss of polarity, especially at the basal layer, but the koilocytic change on the surface is still present. It is distinguished by aberrant cells that have coarse chromatin, irregular nuclear membranes, and high N:C ratios (at least 1:1). There are also visible mitotic figures.[6,7]

CIN3/HSIL:

The upper sections of the epithelium display a substantially greater N:C ratio than in LSIL and HSIL in CIN3 full-thickness atypia, where the base of the lesion is frequently indistinguishable from the surface and mitotic activity may be seen throughout the epithelium (CIN 2). Additionally observed are cell crowding, loss of polarity in the basal layer, an irregular nuclear contour, and coarse chromatin.[6,7]

The following table compares the 3 various classifications for precursor lesions of cervix.

TABLE-1: Classification Of Precursor Lesions Of Cervix

Older classification	WHO classification	Bethesda system
Mild dysplasia	CIN 1	Low grade squamous intraepithelial lesion (LSIL)
Moderate dysplasia	CIN 2	High grade squamous intraepithelial lesion (HSIL)
Severe dysplasia/ Carcinoma in situ	CIN 3	HSIL

"INVASIVE SQUAMOUS CELL CARCINOMA"

General features

The majority of countries still list invasive squamous cell carcinoma of the cervix as the most frequent malignant tumour of the female genital tract and the most common neoplasm among women. Cervical cancer is actually distinct from other types of cancer in humans because it was the first to be directly linked to the effects of an infectious agent, as was already mentioned. This remarkable discovery, for which Harald zur Hausen was rightfully given the Nobel Prize in 2008, carries the possibility of preventing cervical cancer on a global scale. However, the fact that only a small percentage of women who have high-risk HPV infections will acquire cervical cancer shows that other variables must also be present. Other viruses might occasionally fill that role. Although it is currently not thought that EBV is a direct cause of cervical cancer, it is statistically known to be connected with the disease. Although there is inconclusive evidence for a direct carcinogenic effect, human T-cell leukaemia virus (HTLV-1) and human immunodeficiency virus (HIV), when present, adversely affect the prognosis of individuals with cervical cancer and may be associated with a quickly progressing clinical course. Factors associated with the host immune system play a role in the evolution of cervical neoplasia and the maintenance of the HPV infection (CIN and invasive carcinoma). Genes on chromosome 6's human leukocyte antigen (HLA) region are linked to a higher chance of developing high-risk HPV's transforming abilities. A 5- to 10-fold greater incidence of cervical neoplasia is linked to immune system impairment, such as immunosuppressant medication in organ transplant recipients and HIV infection.

The WHO classifies invasive cervical SCCs into eight subtypes based on morphological appearance:

- Keratinising SCC
- Non Keratinising SCC
- Basaloid SCC
- Warty SCC
- Papillary SCC
- Lymphoepithelioma-like SCC
- Squamotransitional Cell
- Verrucous SCC

Among all the above mentioned subtypes, keratinizing, large cell non-keratinizing, and basaloid SCC are the three major subtypes most commonly seen whereas papillary SCC and lymphoepithelial-like SCC are rare patterns. Except Verrucous SCC, all the subtypes are associated with high risk HPV.[7]

CLASSIFICATION:

Keratinizing SCC: These can be of any grade and contain plenty of keratohyaline granules and keratin pearls. The nuclei typically lack the plainly visible nucleoli found in non-keratinizing carcinomas and are big, hyperchromatic, and coarse in chromatin. In its very early stages, keratinizing carcinoma may be more likely to be found in the ectocervix and may have some link with keratinizing SIL as a precursor. Additionally, cytological overlap with HSIL and related surface keratotic reactions may obscure them in cytological preparations.[6]

Non-keratinizing SCC: These are made up of sheets or nests of polygonal squamous cells that may have intercellular bridges but lack keratin pearls. Higher grade tumours with multiple mitotic figures have increased cellular and nuclear pleomorphism. The nuclei are conspicuous, typically irregular or numerous, with chromatin that is coarsely granular..[6]

Basaloid (squamous cell) carcinoma- A significant peripheral palisading, an infiltrative growth pattern, and a limited stromal reactivity are the hallmarks of basaloid (squamous cell) cancer. This tumor exhibits aggressive behavior, as does the corresponding malignancy in the upper aerodigestive tract.[7]

Verrucous carcinoma -A highly differentiated form of squamous cell carcinoma, verrucous carcinoma has a polypoid pattern of growth, an extraordinarily well-differentiated cytologic appearance, and the ability to invade locally but not metastatically. Both condyloma acuminatum and regular squamous cell carcinoma with a conspicuous papillary pattern of development should be recognised from verrucous carcinoma.[7]

ADENOCARCINOMA

Morphology and other features

Of all cervical carcinomas, primary adenocarcinomas account for 5–15% of cases. This percentage is higher among Jewish women, and it has been hypothesised that it is becoming more prevalent overall, especially among young women. Others dispute the link that has been discovered between long-term oral contraceptive use and the emergence of endocervical neoplasia in young patients. They are more challenging to identify on Pap smears than cervical squamous cell carcinomas due to their topography. There are no obvious physical characteristics of the tumour. Microscopically, a well-differentiated glandular pattern with mucin secretion, some of which can leak into the stroma, is the most typical pattern.[8]

IMMUNOHISTOCHEMISTRY (IHC)

Basic Principles of Immunohistochemistry

A technique for recognizing particular antigens in tissues or cells based on antigen-antibody recognition is known as immunohistochemistry (IHC), or immunocytochemistry. It aims to take advantage of the specificity offered by the interaction of an antibody with its antigen at a light-microscopy level. Since Coons initially created an immunofluorescence method to find matching antigens in frozen tissue slices more than 70 years ago, IHC has a lengthy history. However, the technique hasn't been widely used in surgical pathology until the early 1990s. The variety of IHC applications currently in use are the result of a sequence of technological advancements. When an appropriate colorogenic substrate system was present, the enzymatic label known as horseradish peroxidase (HRP), created by Avrameas and Nakane and associates, enabled for the viewing of the labelled antibody by light microscopy. The first successful demonstration of antigens in regularly processed formalin-fixed paraffin-embedded (FFPE) tissues was created by Taylor and Burns at Oxford.[9]

Keratins, CEA, p63 (a homologue of p53 selectively expressed in basal and immature cervical squamous epithelium), and blood type antigens are all expressed immunohistochemically in squamous cell carcinomas of the cervix. Depending on the subtype, the variety of keratins detected in the tumour varies significantly, although it is fairly broad. In addition, there may be reactivity for cathepsin B, ß-hCG, and the parathyroid hormone-related gene. Positivity for HIK1083, CK7 and MUC6 are expressed immunohistochemically in Adenocarcinoma.[6]

COX 2 AND CERVICAL CANCER

Chronic HPV infection in the cervical epithelium results in increased cell turnover and inflammatory mediators, in addition to direct impacts on epithelial DNA: processes that foster the development of cancer. The epidermal growth factor receptor (EGFR) signalling pathway is thought to be responsible for the rise in COX-2 in squamous cell cancer lines, according to research by Dannenburg et al.[10]

Additionally, a recent study shows that EGFR signalling is used by human papillomavirus type 16 oncoproteins E6 and E7 to control COX-2 transcription. These findings show an association between cox-2 overexpression and HPV-related squamous cell malignancies. Tumor Necrosis Factor – α (TNF - α) is activated in the presence of cellular injury leading to increased expression of genes for proinflammatory enzymes. Through a positive feedback loop, this activation increases TNF- α and triggers COX-2 synthesis in epithelial cells. Arachidonic acid in the cytoplasmic membrane is transformed by COX-2 into prostaglandin H-2 and then prostaglandin E-2.

In studies on human cervical tissue, PGE2-mediated inflammatory alterations have been demonstrated. When indomethacin was added, peripheral monocytes' enhanced synthesis of PGE2 and lower levels of interleukin-1 (IL-1) and interferon-gamma (IFN-) were reversed. The extracellular milieu modifications reduce the cell-mediated immune response required to eliminate HPV-transformed cells. Additionally, the proinflammatory effect gets worse as the condition progresses.

Studies further demonstrate the importance of COX-2 expression in the control of apoptosis, the course of disease, neoangiogenesis, and treatment responsiveness. In cases of esophageal squamous neoplasia that were more severe, Liu et al. showed an increase in COX-2

expression.[12] In particular, cervical intraepithelial neoplasia (CIN) and cervical carcinoma were the focus of Dai et al's investigation into the expression of cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), and prostaglandins (PGs) in cervical tissues of different pathological types and their potential roles in the development of cancer. There was a close association between COX-2 and PGs, as evidenced by the overall positive expression of COX-2 and the quantity of PGs, particularly PGE2, in inflammation, CIN, and cervical cancer being higher and significantly higher than that in normal cervix (P 0.001).[11]

diameter (90.9 percent and 72.7 percent, respectively) than in cases with smaller tumours (86.2 percent , 51.7 percent). According to the findings of a study conducted by Dai et al, the COX-2 gene likely plays a role in cervical cancer carcinogenesis by increasing PGs and accelerating tumour growth by increasing PGs and VEGF. As a result, PG expression testing may serve as a prognostic indicator for clinical diagnosis.[11]

In stage Ib cervical cancer patients, Manchana et al. assessed the prevalence of COX-2, connection with numerous clinicopathological variables, and prognostic importance of COX-2. The study demonstrated a substantial link between cervical adenocarcinoma and lymph node metastases, however it was unable to provide a predictive factor for stage Ib cervical cancer.[13]

Bandyopadhyay et al. examined 70 cervical cancer patients. According to the study's findings, COX2 is expressed more frequently in invasive carcinoma cases than in in-situ instances. It is necessary to research COX-2 inhibitors as a therapeutic augmentation for the management of cervical cancer.[4]

As per the study by Hoelle F *et al*. in which they analyzed the effect of COX-2 expression in invasive squamous cell carcinoma of cervix revealed that positive immunohistochemistry (IHC) staining was detected in 23% of patients with a higher percentage of staining in tumour

cells as compared to peritumoural stromal cells. In this study, it was demonstrated that COX-2 expression was significantly associated with lymphovascular invasion.[2]

A study conducted by Kim Y *et al.* on 105 uterine cervical cancer patients with stage-IIB showed COX-2 expression in adenocarcinoma patients was higher than squamous cell carcinoma patients(57% vs. 24%), leading to poor prognosis irrespective of its histological type. In this they concluded that patients having positivity for COX-2 were most reliable in conducting trials of selective COX-2 inhibitor adjunctive therapy.[3]

Patients with clinically confirmed lymph node enlargement were found to have increased COX2 expression. With a p-value of 0.026, it was concluded that this was statistically significant. The Miaoling et al. study lends weight to this.[14]

MATERIALS AND METHODS

SOURCE OF DATA

Cervical biopsies and hysterectomy specimens received in the histopathology section in the department of pathology, "BLDE (Deemed To Be University) Shri B.M. Patil Medical College, Hospital and Research Centre", Vijayapura.

STUDY PERIOD:

January 2020 to July 2022 (8months of retrospective and 1year 11months of prospective study).

INCLUSION CRITERIA:

Histologically diagnosed cases of in-situ and malignant lesions of the cervix were included.

EXCLUSION CRITERIA:

Patients who were on treatment with chemotherapy or radiotherapy prior to surgery were excluded.

SAMPLE COLLECTION:

Paraffin blocks of histologically diagnosed cases of carcinoma of the cervix (in-situ, squamous cell carcinoma, and adenocarcinoma) will be retrieved from the Histopathology Section, Department of Pathology, from 1st January 2020 to 31st July 2022. All Clinical details, findings on imaging, if any, were recorded.

METHODS OF COLLECTION OF DATA:

Tissue for the study included were cervical biopsy and hysterectomy specimens. The tissue was preserved in 10 % buffered formalin and processed routinely. Three 4 micron-thick sections were prepared from each tissue block. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis. The histologic grade, depth of invasion, and presence of lymphovascular invasion were noted.

Two sections were mounted on poly L lysine coated slides, which were subjected to COX-2 immunohistochemical staining. Immunohistochemistry for COX-2 was performed using a rabbit monoclonal antibody to COX-2 and a supersensitive polymer-based detection system (Biogenex). Formalin-fixed, paraffin-embedded tissue sections were used for staining. After deparaffinization, tissue sections were rehydrated using descending grades of alcohol and water. Antigen retrieval was done by heat treatment using a microwave oven. Tris buffer saline (TBS) was used for washing. Peroxide blocking was done for 10 minutes. This was followed by a power block for 10 minutes, cleaning, and incubation with primary antibody for one hour at 4°C. Slides were rewashed in TBS and incubated with a secondary antibody for 30 minutes. After washing and cleaning, the link label will be added and incubated for 30 minutes.

After washing in the buffer, DAB was added and incubated for 10 minutes. After washing, slides were counterstained with Hematoxylin. Cases of colon carcinoma were taken as positive controls. Cytoplasmic and membrane staining of tumour cells were considered as positive staining.

Positive staining patterns was graded as undetected, low (expressed in 10% tumour cells), moderate (10-50% positive tumour cells), and high (>50% positive tumour cells). Statistical analysis was performed with Fisher's two-tailed exact test (with Freeman Halton extension).

For the statistical purpose, the expression pattern was classified as expressed (including low, moderate, and high positivity) and not expressed (including the undetected cases).

STATISTICAL ANALYSIS

The data obtained was entered in a Microsoft excel sheet, and statistical analysis was done by using statistical package for the social sciences (Version 20)

For normally distributed continuous variables was compared using Independent test. For not normally distributed variables Mann Whitney U test was used. Categorical variables were compared using Chi square test.

Association between variables was analyzed using correlation, scattered diagram (Quantitate data) and Chi square test (Qualitative data)

P value of < 0.05 was considered statistically significant. All statistical tests were performed two tailed.

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34.319[a]	15	0.003
Likelihood Ratio	38.867	15	0.001
N of Valid Cases	62		

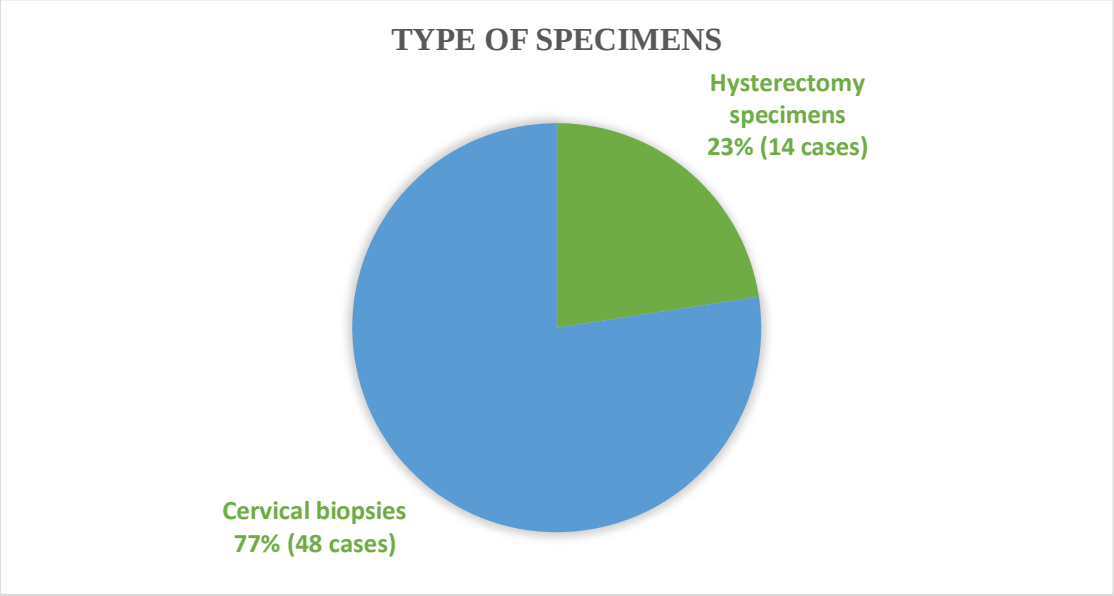
OBSERVATION AND RESULTS

Study was done on total 62 specimens. Out of 62 specimens 14 were hysterectomy specimens and 48 were cervical biopsies from 1st January 2020 to 31st July 2022 in the Department of Pathology. H&E staining was done on all slides. Cox-2 IHC staining was done on slides of cervical cancer.

TYPE OF SPECIMENS

Out of 62 cases, 14 were hysterectomy specimens and 48 were cervical biopsies(Fig 5).

FIGURE 5: TYPE OF SPECIMENS



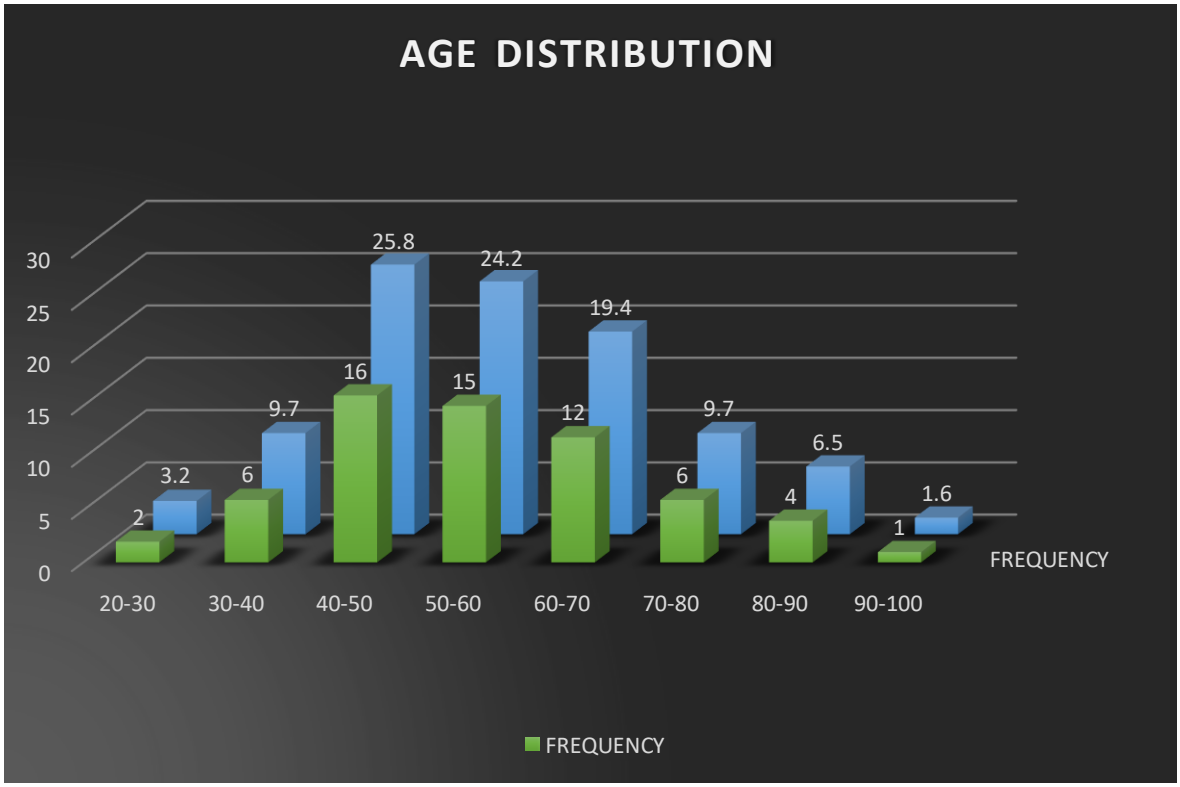
AGE DISTRIBUTION IN ALL CASES

Among all the patients (N = 62) in the study, the majority of patients were in age group 40 to 50 years comprising of 16 cases (25.8% of study population). The detailed representation is shown below (Table-2 & Fig 6).

TABLE-2: AGE DISTRIBUTION IN ALL CASES

AGE (YEARS)	NO. OF PATIENTS	PERCENTAGE (%)
20-30	2	3.2
30-40	6	9.7
40-50	16	25.8
50-60	15	24.2
60-70	12	19.4
70-80	6	9.7
80-90	4	6.5
90-100	1	1.6
Total	**62**	**100.0**

Fig. 6- Distribution of patients according to Age



MEAN AGE DISTRIBUTION IN ALL CASES

In this study, the minimum age was 22 years and maximum was 90 years and the mean age of presentation in this study was 54.61 years.(Table-3)

TABLE-3 : MEAN AGE DISTRIBUTION IN ALL CASES

MEASURES OF CENTRAL TENDENCY	AGE IN YEARS
Minimum	22
Maximum	90
Mean	54.61
Std. Deviation	15.096

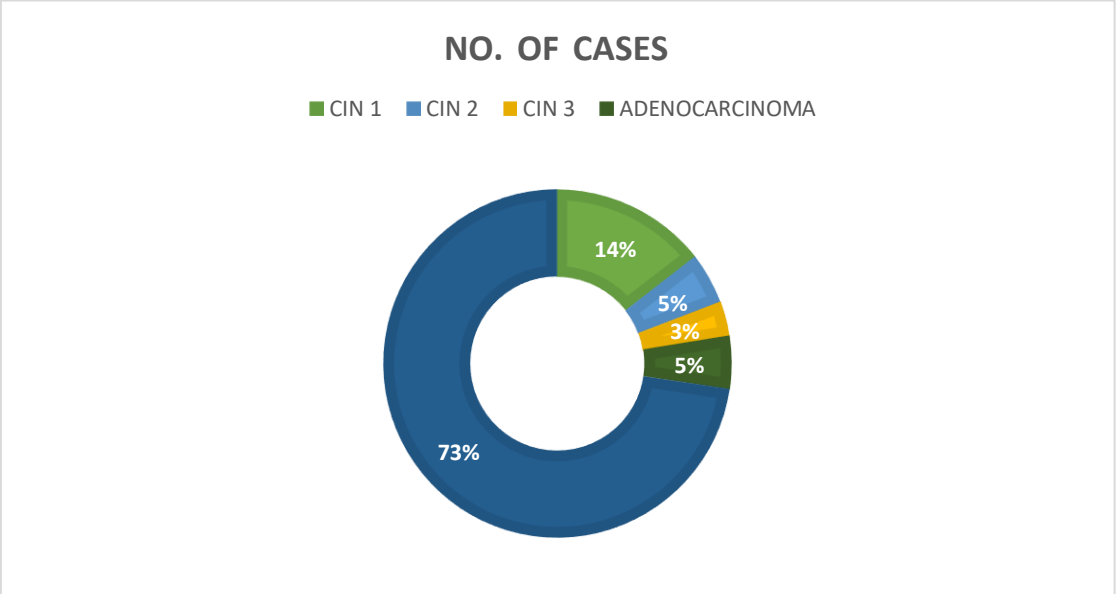
DISTRIBUTION OF PATIENTS ACCORDING TO HISTOLOGICAL DIAGNOSIS

Among all 62 cases included in this study, 45 (72.6%) cases of Squamous cell carcinoma, 9 (14.5%) cases of CIN 1, 3 (4.8%) cases of CIN 2, 2 (3.2%) cases of CIN 3, and 3 (4.8%) cases of Adenocarcinoma were reported (Table-4 & Fig-7).

TABLE-4: DISTRIBUTION OF PATIENTS ACCORDING TO HISTOLOGICAL DIAGNOSIS

HISTOLOGIC TYPE	NO. OF CASES	PERCENT
SQUAMOUS CELL CARCINOMA	45	72.6
CIN 1	9	14.5
CIN 2	3	4.8
CIN 3	2	3.2
ADENOCARCINOMA	3	4.8
TOTAL	**62**	**100.0**

Fig. 7- Distribution of patients according to Histological diagnosis



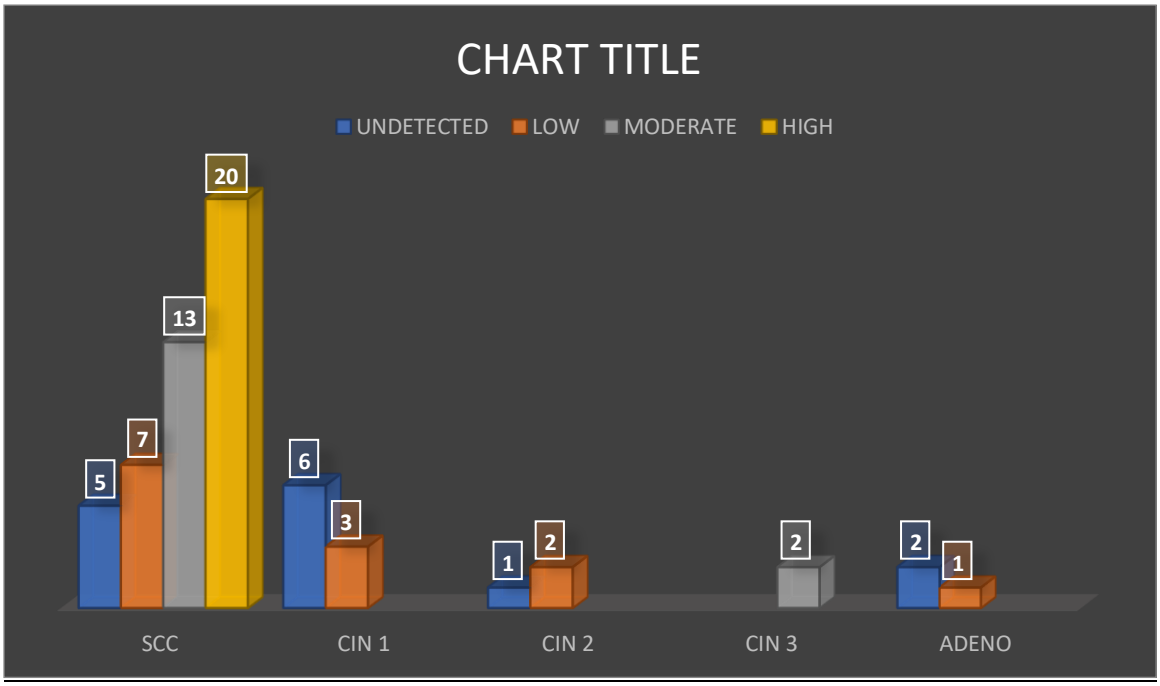
EXPRESSION OF COX-2 WITH TUMOR DIFFERENTIATION

Among all 62 cases included in this study, 45 (72.6%) cases of Squamous cell carcinoma cases were reported. Out of these 45 cases 20 (32.3%) cases showed higher expression (>50% positive tumour cells) of COX-2, when compared with others all cases (3 out of 9 cases showed low expression in CIN1, 2 out of 3 cases showed low expression in CIN2, 2 out of 2 cases showed moderate expression in CIN3 and 1 out of 3 cases showed low expression in Adenocarcinoma) as shown in Table-5 & Fig- 8.

TABLE-5 : EXPRESSION OF COX-2 WITH TUMOR DIFFERENTIATION

HISTOLOGIC GRADE	UNDETECTED	LOW EXPRESSION	MODERATE EXPRESSION	HIGH EXPRESSION	PERCENTAGE (%)	P- Value
SCC	5 (8.1%)	7 (11.3%)	13 (21.0%)	20 (32.3%)	72.7	0.003
CIN 1	6 (9.7%)	3 (4.8%)	0.0	0.0	14.5	
CIN 2	1 (1.6%)	2 (3.2%)	0.0	0.0	4.8	
CIN 3	0.0	0.0	2 (3.2%)	0.0	3.2	
ACC	2 (3.2%)	1 (1.6%)	0.0	0.0	4.8	

Fig. 8- Expression of COX 2 with tumor differentiation



MICROSCOPIC IMAGES- IHC SLIDES :

Fig. 9- Undetected grading of COX 2

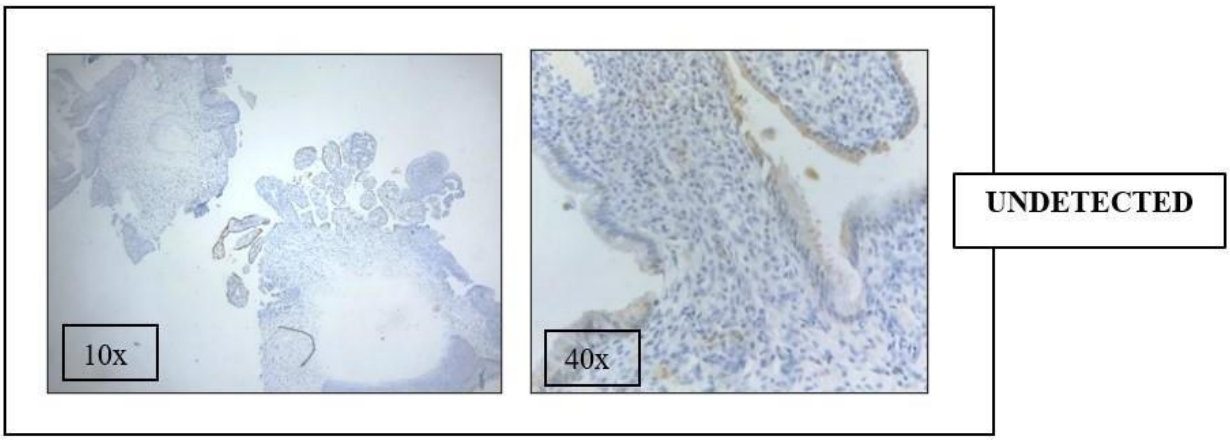


Fig. 10- Low expression of COX 2

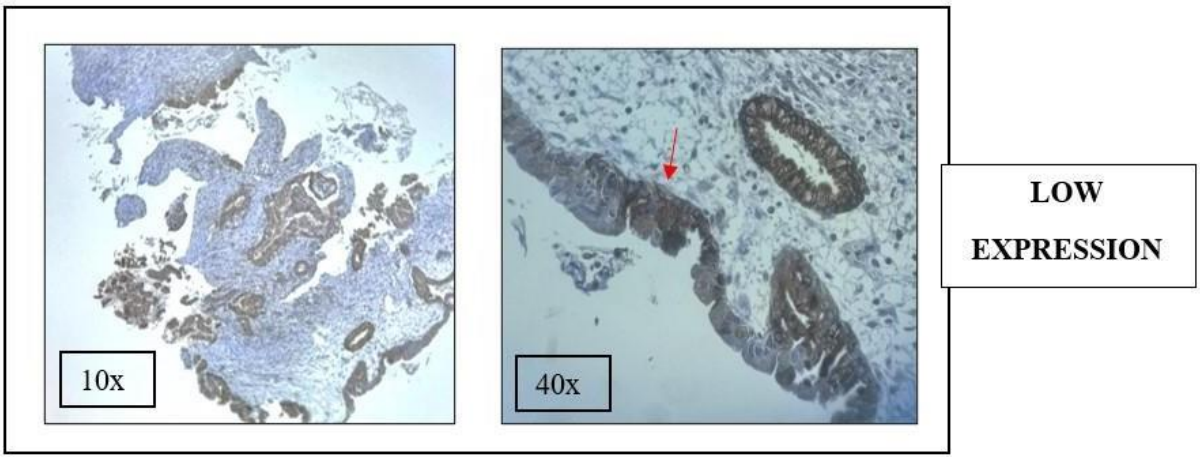


Fig. 11- Moderate expression of COX 2

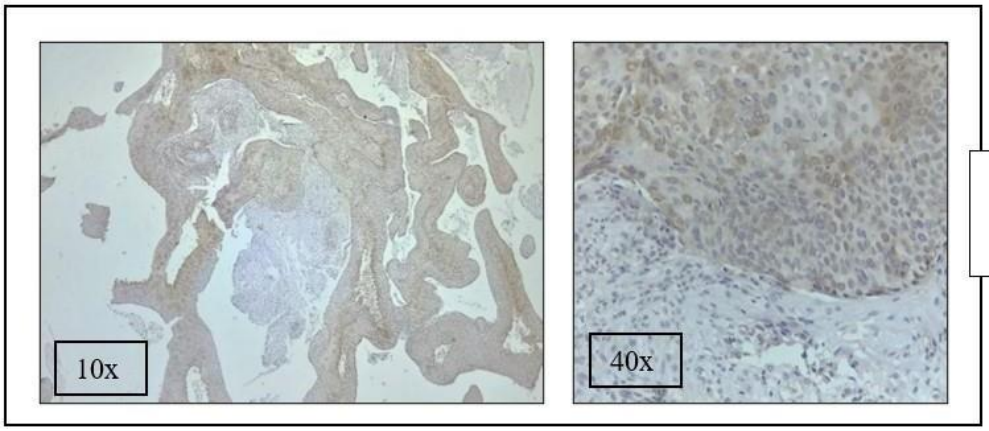


Fig. 12- High expression of COX 2

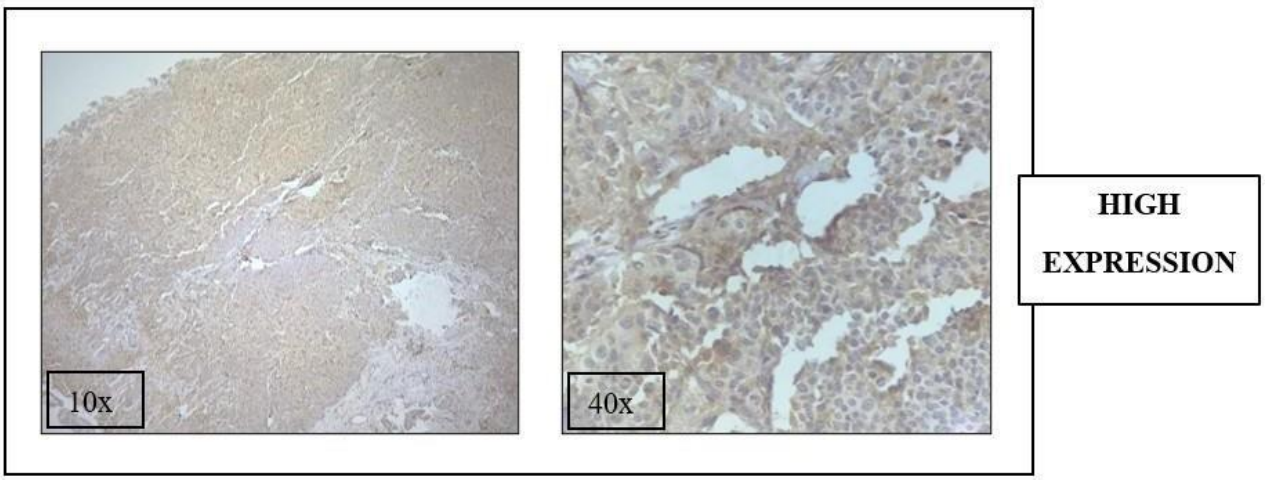
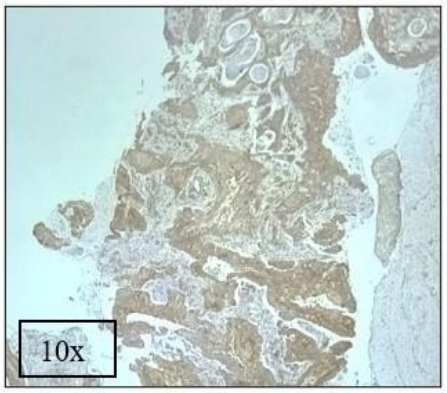
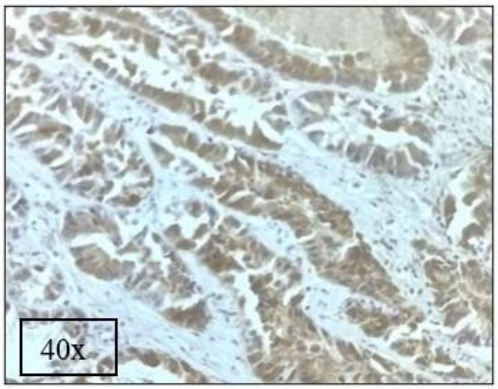


Fig. 13- Colon Carcinoma- Control slide for COX 2





CONTROL SLIDE

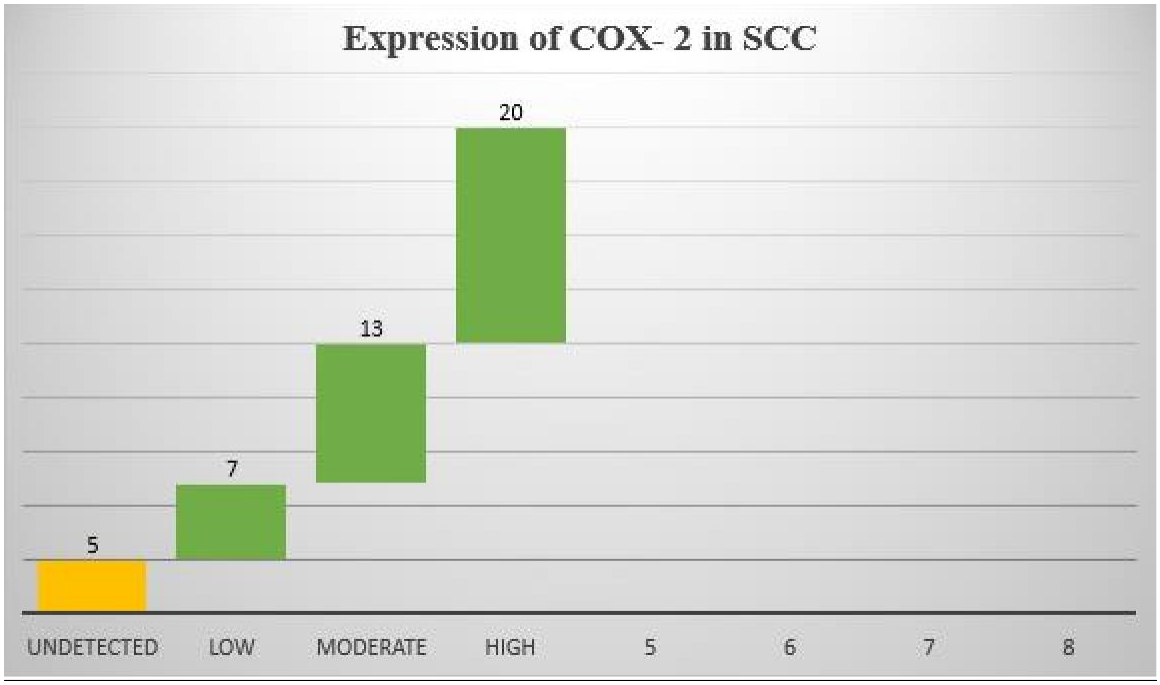
EXPRESSION OF COX-2 IN SQUAMOUS CELL CARCINOMA CASES

Among 45 cases of SCC, COX-2 expression was undetected in 5 cases, 7 cases showed low expression (positivity in 10% tumour cells) 13 cases showed moderate expression (positivity in 10-50% tumour cells) and 20 cases showed high expression (positivity in >50% tumour cells)(Table-6 & Fig 14).

TABLE-6 : EXPRESSION OF COX-2 IN SQUAMOUS CELL CARCINOMA CASES

	FREQUENCY	PERCENT
UNDETECTED	5	11.1
LOW	7	15.5
MODERATE	13	28.8
HIGH	20	44.4

Fig. 14- Expression of COX 2 in SCC



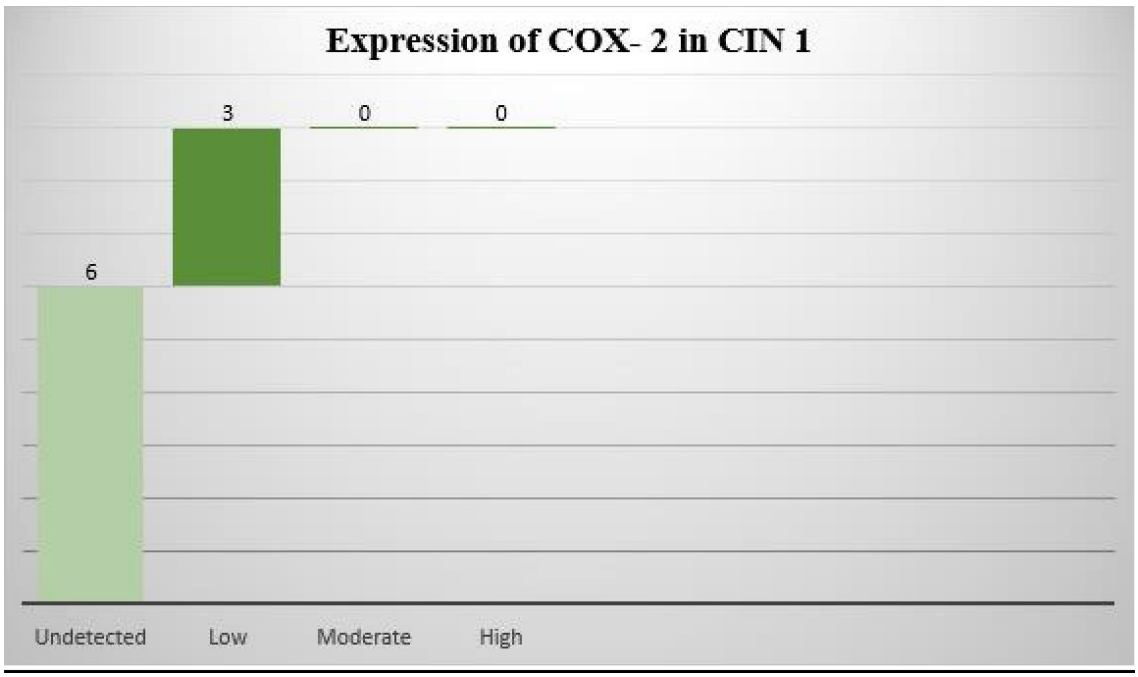
EXPRESSION OF COX-2 IN CIN 1 CASES

Out of 9 cases of CIN 1, 3 were showing low (positivity in 10% tumour cells) expression as shown in Table-7 & Fig 15.

TABLE-7 : EXPRESSION OF COX-2 IN CIN 1 CASES

	FREQUENCY	PERCENT
UNDETECTED	6	66.6
LOW	3	33.3
MODERATE	0	0
HIGH	0	0

Fig. 15- Expression of COX 2 in CIN 1



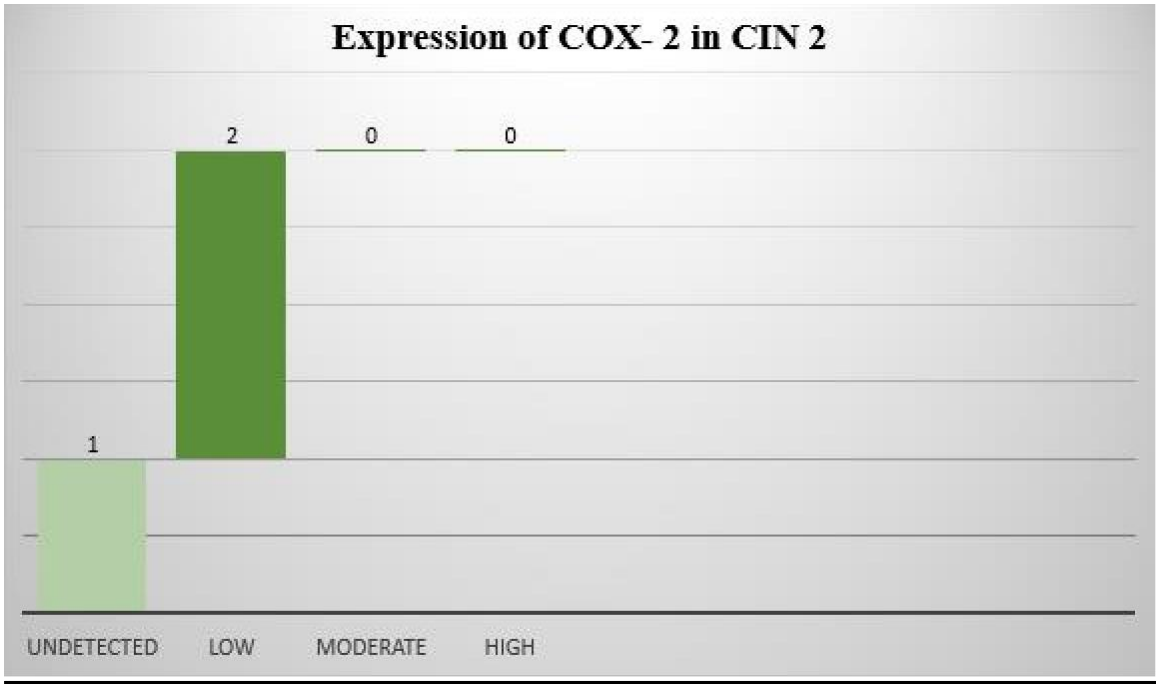
EXPRESSION OF COX-2 IN CIN 2 CASES

Out of 3 cases of CIN 2, 2 were showing low (positivity in 10% tumour cells) expression as shown in Table-8 & Fig 16.

TABLE-8 : EXPRESSION OF COX-2 IN CIN 2 CASES

	FREQUENCY	PERCENT
UNDETECTED	1	33.3
LOW	2	66.6
MODERATE	0	0
HIGH	0	0

Fig. 16- Expression of COX 2 in CIN 2



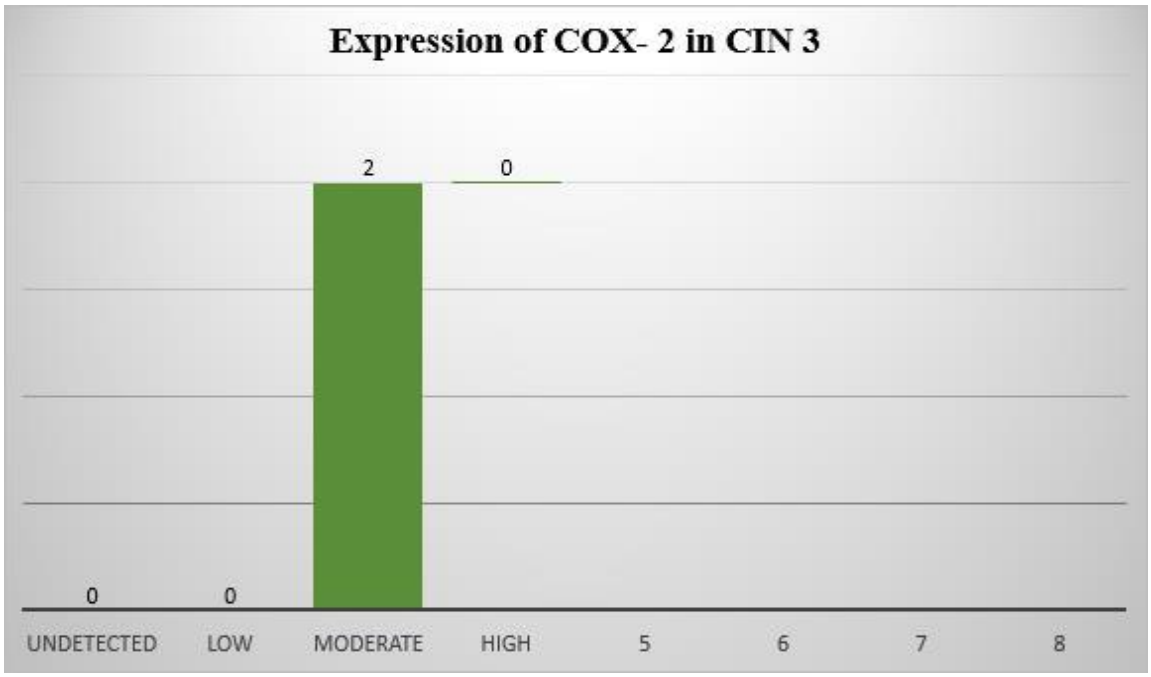
EXPRESSION OF COX-2 IN CIN 3 CASES

2 out of 2 cases of CIN 3, were showing moderate expression of COX-2 as shown in Table-9 & Fig 17.

TABLE-9 : EXPRESSION OF COX-2 IN CIN 3 CASES

	FREQUENCY	PERCENT
UNDETECTED	2	0
LOW	0	0
MODERATE	2	100
HIGH	0	0

Fig. 17- Expression of COX 2 in CIN 3



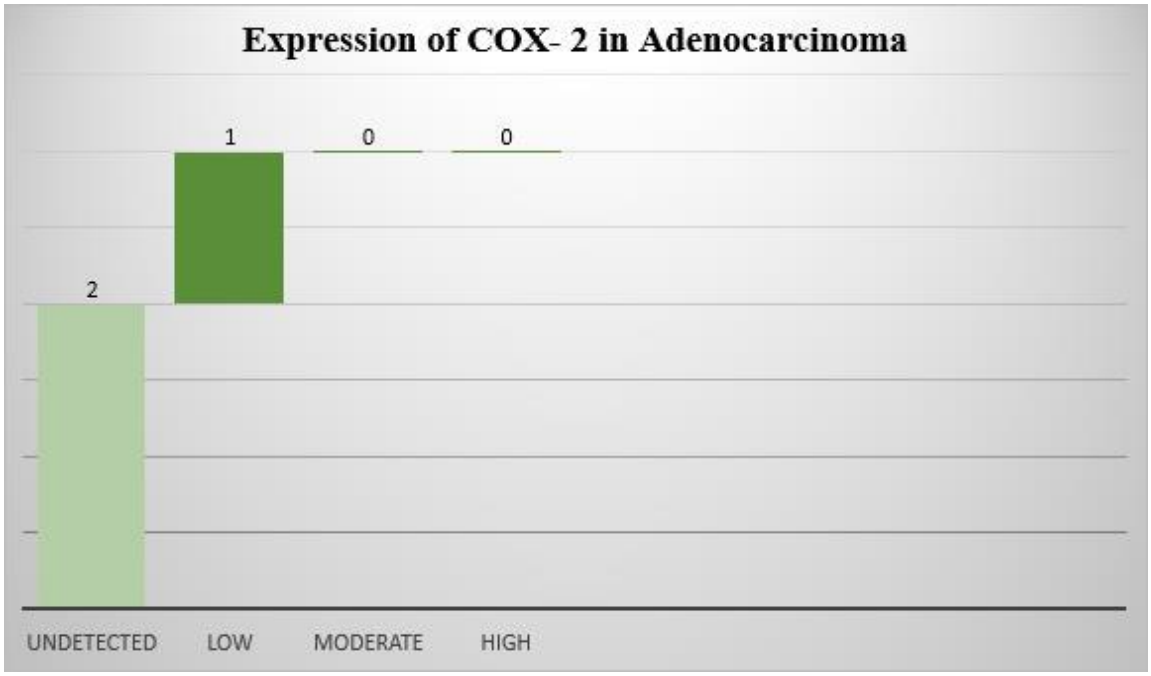
EXPRESSION OF COX-2 IN ADENOCARCINOMA CASES

Out of 3 cases of Adenocarcinoma, 2 were undetected and 1 case showed low (positivity in 10% tumour cells) expression .(Table-10 & Fig 18)

TABLE-10 : EXPRESSION OF COX-2 IN ADENOCARCINOMA CASES

	FREQUENCY	PERCENT
UNDETECTED	2	66.6
LOW	1	33.3
MODERATE	0	0
HIGH	0	0

Fig. 18- Expression of COX 2 in Adenocarcimona



PARAMETRIAL AND LYMPHOVASCULAR INVASION

Out of the 62 cases, 48 cases were cervical biopsy and 14 cases were hysterectomy specimens. Out of 14 hysterectomy cases, none of the case showed parametrial and lymphovascular invasion.

DISCUSSION

Cyclooxygenase, the primary enzyme in prostaglandin metabolism, has undergone extensive research to determine its potential role in the initiation and progression of tumors. It is well known that the concentration of prostaglandin in tumor tissues is significantly higher than that in the corresponding normal tissues. Both in the early and late stages of carcinogenesis, COX-2 expression is found to have significant involvement. As COX-2 is present in cells independently during the initial stages of cell differentiation or replication, changes in COX-2 expression have been seen more commonly in a variety of malignant cancers. Adenomatous and metaplastic lesions of the stomach, pre-neoplastic lesions of the lung, and pre-invasive neoplasias of the breast, bladder, and pancreas have all been shown to have increased COX-2 expression.[4] Studies on the expression of COX-2 in cervical cancer are rare. It has been demonstrated that it is expressed in invasive carcinomas and dysplastic epithelium.

CORRELATION OF COX 2 WITH HISTOLOGICAL TYPE OF CERVICAL CANCER

Increased expression of COX 2 has been shown to be associated with increased severity of dysplasia . In the present study COX 2 expression was lower in in-situ carcinomas compared to invasive squamous cell carcinomas. The results were statistically significant with p value < 0.05. Bandhyopadhyay et al also reported significant difference in cox 2 expression pattern between in-situ CIN and invasive SCC with the expression being more in invasive cases (p=0.002).[4] According to Balan et al. the intensity of cytoplasmic COX-2 immunostaining had a weaker expression in specimens with LSIL and stronger one in those diagnosed as

HSIL.[15] Similarly according to Fukazawa et al the mean COX 2 expression was predominatly cytoplasmic, increasing significantly from CIN 1 to CIN 2, CIN 3 and SCC ($p<0.001$).[16]

CORRELATION OF INTENSITY OF COX-2 EXPRESSION WITH TUMOUR GRADE.

In the present study we noted that COX-2 staining intensity increased with the grade of the tumour. Highest intensity was seen squamous cell carcinomas when compared to cervical intraepithelial lesions. These results were statistically significant with p value of 0.003. Similar results were shown by Balan et al and Fukazawa et al.[15,16] But according to Bandhyopadhay et al these results were not statistically significant.[4]

CORRELATION OF INTENSITY OF COX-2 EXPRESION WITH SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA.

Cervical adenocarcinomas have also been shown to express COX-2. As contrast to squamous cell carcinoma, cervix adenocarcinomas in the current study revealed a low positivity in one case out of 3 cases. This variation was not found to be statistically significant. It's possible that there weren't enough cases to make any conclusions from this. According to Manchana et al. COX-2 expression in cervical adenocarcinoma was higher than in squamous cell carcinoma.[13] The study found a significant relationship between Cox-2 expression and cervical adenocarcinoma. Fathima et al.[17] evaluated 130 cervical cancer patients for COX-2 and EGFR expression. Adenocarcinomas (ACC) had higher levels of COX-2 expression than Adenosquamous carcinomas (ASC).

A study done by Khunamornpong S et al. has demonstrated that adenocarcinomas with strong COX-2 expression, shows unfavourable therapeutic response[18]. A study done by Kim Y *et al.*, concluded that patients having positivity for COX-2 were most reliable in conducting trials of selective COX-2 inhibitor adjunctive therapy.[3]

TABLE 11- CORRELATION OF COX-2 EXPRESSION WITH DIFFERENT TYPES OF CERVICAL CANCER WITH OTHER STUDIES.

STUDY	TOTAL CASES	SCC	ADENOCARCINOMA
Present study	62	40 out of 45 were positive	1 out of 3 was positive
Kulkarni S et al, (2001)[1]	13	8 out of 9 were positive	2 out of 2 were positive
Kim Y *et al* (2004)[3]	105	20 (24%) out of 84 were positive	12 (57%) out of 21 were positive
Ferrandina G *et al* (2004)	99	41 (50.6) out of 81	12 out of 12 (100%)

CORRELATION OF INTENSITY OF COX-2 EXPRESSION WITH LYMPHOVASCULAR INVASION & PARAMATRIAL INVASION.

In the present study out of 14 hysterectomy cases, no lymphovascular and parametrial invasion was noted. So, correlation of COX 2 expression with lymphovascular and parametrial invasion cannot be commented. However, a study by Khunamornpong et al. with sample size of 196 cases found that lympho vascular space invasion was associated with COX-2 expression and lymph node metastasis (p=0.007) in cervical SCC.[18] They came to the conclusion that COX-2 expression could facilitate lymph node metastases following lymphovascular space invasion. A similar study was performed by Mandic et al. Also, the correlation between COX-2 expression and the presence of lymphatic invasion (LVI) showed a statistically significant difference (61.9% LVI-positive, LVI-negative-33.3%, p = 0.02).[19]

This finding once more emphasizes the potential predictive value of COX-2 expression, which may be combined with other important variables to adjust postoperative adjuvant therapy in SCC.[20,21,22]

SUMMARY

- This study was done at the Department of Pathology, "B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre", Vijayapura, Karnataka.
- Cervical biopsies and hysterectomy specimens received in the histopathology section for routine histopathological examination from January 2020 to July 2022 (8months of retrospective and 1year 11months of prospective study) in the department of pathology, BLDE (Deemed To Be University) Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura.
- Hematoxylin and Eosin was used to stain formalin-fixed paraffin-embedded tissue sections.
- The histologic type of tumour, parametrial involvement and lymphovascular space invasion were noted.
- Using a rabbit monoclonal antibody for the COX-2 supersensitive polymer-based detection method, immunohistochemistry for COX-2 was carried out (Biogenex).
- Each batch of slides was subjected to positive and negative controls.
- Cases of colon carcinoma were taken as positive controls.
- Microscopically, IHC slides of COX 2 were assessed. Cytoplasmic and membrane staining of tumour cells was considered as positive staining.
- The total number of tumour cells expressing COX2 was calculated by counting the number of tumour cells in each high-power field (400x).

- Positive staining patterns were graded as undetected, low (expressed in 10% tumour cells), moderate (10-50% positive tumour cells), and high (>50% positive tumour cells).
- Out of 62 cases 14 were hysterectomy specimens and 48 were cervical biopsies
- In the present study the minimum age of presentation was 22 years maximum age was 90 years with a mean of 54.61 years and standard deviation of 15.096.
- Among all 62 cases included in this study, 45 (72.6%) cases of Squamous cell carcinoma cases were reported. Out of these 45 cases 20 (32.3%) cases showed higher expression (>50% positive tumour cells) of COX-2, when compared with others all cases (3 out of 9 cases showed low expression in CIN1, 2 out of 3 cases showed low expression in CIN2, 2 out of 2 cases showed moderate expression in CIN3 and 1 out of 3 cases showed low expression in Adenocarcinoma.
- Out of 14 cases, lymphovascular and parametrial invasion was not seen in any case.
- In the presence study, in-situ carcinomas had lower COX 2 expression than invasive carcinomas. With a p value of 0.003, the results were statistically significant.
- The current study found that COX-2 staining intensity increased as tumor grade increased. With a p value of 0.003, these findings were statistically significant.

CONCLUSION

- The immunohistochemistry profile with multiple markers will aid in the diagnosis and comprehension of the histogenesis of the tumour because carcinoma of the cervix is one of the most common tumours affecting women. The same will be useful in designing the patients' therapy schedule.
- The high expression of COX-2 in a variety of cervical neoplasms, including squamous cell carcinoma, CIN and adenocarcinoma of the cervix, suggests that COX-2 expression may be clinically related to the initiation and progression of cervical carcinoma.
- Our study shows that the intensity of COX-2 expression increased along with the severity of cervical dysplasia from CIN to invasive cancer.
- Cervical squamous cell carcinomas showed strong positivity for COX-2 with intense staining pattern compared to adenocarcinomas. To assess the function of COX-2 in cervical adenocarcinomas and the possible function of COX-2 inhibitors in such situations, studies with a bigger sample size are necessary.
- In present study, expression of COX-2 correlation with parametrial and lymphovascular invasion cannot be commented due to absence of parametrial and lymphovascular invasion in 14 cases and less sample size
- As the sample size was limited, in our study less CIN and Adenocarcinoma cases were taken when compared with SCC. So appropriate results was not obtained.

REFERENCES

1. Kulkarni S, S. Rader J, Zhang F. Cyclooxygenase-2 Is Overexpressed in Human Cervical Cancer. American Association for Cancer Research. 2001;7:429-434.
2. Hoellen F, Waldmann A, Banz-Jansen C, Rody A, Heide M, Köster F et al. Expression of cyclooxygenase-2 in cervical cancer is associated with lymphovascular invasion. Oncology Letters. 2016;12(4):2351-2356.
3. Kim Y, Kim G, Pyo H, Cho N, Keum K, Lee C et al. Differential cyclooxygenase-2 expression in squamous cell carcinoma and adenocarcinoma of the uterine cervix. International Journal of Radiation Oncology*Biology*Physics. 2004;60(3):822-829.
4. Bandyopadhyay R, Chatterjee U, Kr. Mondal S, Nag D, Kr. Sinha S. A study on the expression pattern of cyclooxygenase-2 in carcinoma of the cervix. Indian Journal of Pathology and Microbiology. 2011;54(4):695-699.
5. Young B, Woodford P, O'Dowd G. Wheater's functional histology E-Book: a text and colour atlas. Elsevier Health Sciences; 2013 Oct 9.
6. McCluggage WG, Singh N, Gilks CB. Key changes to the World Health Organization (WHO) classification of female genital tumours introduced in the 5th edition (2020). Histopathology. 2022 Apr;80(5):762-78.
7. Sternberg SS, Mills SE, Carter D, editors. Sternberg's diagnostic surgical pathology. Lippincott Williams & Wilkins; 2022
8. Rosai J. Rosai and Ackerman's surgical pathology e-book. Elsevier Health Sciences; 2011 Jun 20.
9. Cina SJ, Richardson MS, Austin RM, Kurman RJ: Immunohistochemical staining for Ki- 67 antigen, carcinoembryonic antigen, and p53 in the differential diagnosis of glandular lesionsof the cervix. *Mod Pathol* 1997; 10:176-180.

10. Lippman SM, Gibson N, Subbaramaiah K, Dannenberg AJ. Combined targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways. Clinical Cancer Research. 2005 Sep 1;11(17):6097-9.

11. Dai Y, Zhang X, Peng Y, Wang Z. The expression of cyclooxygenase-2, VEGF and PGs in CIN and cervical carcinoma. Gynecologic oncology. 2005 Apr 30;97(1):96-103.

12. Huang M, Chen Q, Xiao J, Liu C, Zhao X. Prognostic significance of cyclooxygenase-2 incervical cancer: A meta-analysis. International journal of cancer. 2013 Jan 15;132(2):363-73.

13. Manchana T, Triratanachat S, Sirisabya N, Vasuratna A, Termrungruanglert W, Tresukosol D. Prevalence and prognostic significance of COX-2 expression in stage IB cervical cancer. Gynecologic oncology. 2006 Mar 31;100(3):556-60.

14. Miaoling Huang, Qing Chen, Jianpeng Xiao, Changhao Liu and Xiaomiao Zhao Prognostic significance of cyclooxygenase-2 in cervical cancer: A meta-analysis Int. J. Cancer: 132, 363–373

15. Balan RA, Amalinei C, Giusca SE, Ditescu D, Gheorghita V, Crauciuc E, Caruntu ID. Immunohistochemical evaluation of COX-2 expression in HPV-positive cervical squamous intraepithelial lesions. Rom J Morphol Embryol. 2011 Jan 1;52(1):39-43.

16. Fukazawa EM, Baiocchi G, Soares FA, Kumagai LY, Faloppa CC, Badiglian-Filho L, Coelho FR, Gonçalves WJ, Costa RL, Góes JC. Cox-2, EGFR, and ERBB-2 expression in cervical intraepithelial neoplasia and cervical cancer using an automated imaging system. International Journal of Gynecological Pathology. 2014 May 1;33(3):225-34.

17. Baltazar F, Longatto Filho A, Pinheiro C, Moreira MA, Queiroz GS, Oton GJ, Júnior AF, Ribeiro LF, Schmitt FC. Cyclooxygenase-2 and epidermal growth factor receptor expressions in different histological subtypes of cervical carcinomas. International journal of gynecological pathology. 2007 Jul 1;26(3):235-41.

18. Khunamornpong S, Settakorn J, Sukpan K, Srisomboon J, Ruangvejvorachai P, Thorner PS,Siriaunkgul S. Cyclooxygenase-2 expression in squamous cell carcinoma of the uterine cervixis associated with lymph node metastasis. Gynecologic oncology. 2009 Jan 31;112(1):241-7.

19. Mandic A, Knezevic S, Kapicl T, Nincic D, Malenkovic G.Cyclooxygenase-2 expression in cervical cancer. Vojnosanit Pregl 2014; 71(11): 997–1005.

20. Crissman JD, Makuch R, Budhraja M. Histopathologic grading of SCC of the uterine cervix. An evaluation of 70 stage Ib patients. Cancer 1985;55:1590-6.

21. Barber BR, Sommers SC, Rotterdam H, Kwon T. Vascular invasion as a prognostic factor in stage lB cancer of the cervix. Obstet Gynecol 1978;52:343-8 67.Kang MK, Park W, Choi Y-L, Cho EY, Ahn G, Nam H, et al. The effect of cyclooxygenase-2 expression on tumor volume response in patients treated with radiotherapy for uterine cervical cancer. J Korean MedSci 2009;24:1170-6.

22. Ryu HS, Chang KH, Yang HW, Kim MS, Kwon HC, Oh KS. High cyclooxygenase-2 expression in Stage IB cervical cancer with lymph node metastasis or parametrial invasion. Gynecol Oncol 2000;76:320-5.

ANNEXURE – I

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/NO-09/2021 Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A study of expression of Cyclooxygen ASE-2 in cervical cancer.

Name of PG student: Dr Nuzhath Shaik, Department of Pathology

Name of Guide/Co-investigator: Dr S M Nerune , Associate Professor of Pathology

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

12

ANNEXURE-II

B.L.D.E (DEEMED TO BE UNIVERSITY) SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYPURA-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: "A STUDY OF EXPRESSION OF CYCLOOXYGENASE-2 IN CERVICAL INTRA EPITHELIAL NEOPLASIA AND CERVICAL CANCER"

PRINCIPAL INVESTIGATOR: DR. NUZHATH SHAIK

P.G. DEPARTMENT OF PATHOLOGY

P.G GUIDE : DR. SAVITRI MALLIKARJUN NERUNE D.C.P DNB. (Path)

ASSOCIATE PROFESSOR, DEPT OF PATHOLOGY.

PURPOSE OF RESEARCH:

I have been informed that the present study is a study of the Expression of COX-2 in premalignant and malignant conditions is essential for therapeutic benefit of the patient.

PROCEDURE:

I understand that I undergo detailed history and after which necessary investigations will be done.

RISK AND DISCOMFORTS:

I understand that, there is no risk involved for me being a part of the study.

BENEFITS:

I understand that my participation in the study will help to know the Efficacy of Platelet and Reticulocyte parameters in early diagnosis of sepsis in Intensive Care Unit patients**.**

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I might be asked for more information about my disease at any time.

REFUSAL FOR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore, I agree to participate in the present study.

_____

Participant/Guardian Date:

_____

Signature of Witness Date:

I have explained the patient/patients attender the purpose of study, the procedure required and possible risk and benefit of my ability in the vernacular language.

_____

Investigator/P.G Date:

_____

Witness to Signature Date:

ANNEXURE - III

PROFORMA

NAME : **OP/IP No. :**

AGE :

SEX : **D.O.A :**

RELIGION : **D.O.D :**

OCCUPATION :

RESIDENCE :

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

Examination finding :

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT :

BIOPSY :

HPR Finding :

Expression of COX-2 in CIN-1, CIN-2, CIN-3, Squamous cell carcinoma and Adenocarinoma given as:

Detected,

Undetected,

low (expressed in 10% tumor cells),

moderate (10-50% positive tumor cells), and

high (>50% positive tumor cells).

Parametrial invasion- Present

Absent

Lymphovascular invasion- Present

Absent.

Pre-malignant or Malignant:

IHC show **:** COX-2 labelling index:

Based on percentage of positively stained tumour cells grade is given as undetected, low (expressed in 10% tumor cells), moderate (10-50% positive tumor cells), and high (>50% positive tumor cells)

Diagnosis **:**

KEY TO MASTER CHART

Sl No.	Serial Number
HPR NO	Histopathology report number
IP NO	Inpatient number
CIN 1	Cervical Intraepithelila Neoplasia -1
CIN 2	Cervical Intraepithelila Neoplasia -2
CIN 3	Cervical Intraepithelila Neoplasia -3
SCC	Squamous Cell Carcinoma
COX 2	Cyclooxygenase 2

MASTER CHART

SL.NO	NAME	AGE/SEX	IP NO.	HPR.NO	HPR IMPRESSION	COX 2 EXPRESSION
1	Kashibai	65yrs/F	43647	2130/21	Moderately differentiated SCC	Undetected
2	Meenakshi Deva Pooje	50yss/F	102489	1886/22	Invasve SCC	Undetected
3	Huseen bi	75yrs/F	139668	2321/20	Moderately differentiated non-keratinizing SCC	Moderate
4	Shivamma Pujari	50yrs/F	15928	993/21/A	SCC-Papillary type	Moderate
5	Sumitra	45yrs/F	1220808	1098/21/B	Moderately differentiated non-keratinizing SCC	Moderate
6	Iramma	88yrs/F	9278	1803/20/A	Non-keratinizing SCC	Moderate
7	Laxmi Bai	35yrs/F	1914	1914/21	Low grade adenocarcinoma	Low
8	Shakuntala	54yrs/F	1783	411/20	Moderately differentiated non-keratinizing SCC	High
9	Neelamma	50yrs/F	128895	1143/21	Villoglandular papillary adenocarcinoma	Undetected
10	Gwalamma	65yrs/F	11233	135/21	Non-keratinizing SCC	Low
11	Gorakka	44yrs/F	133357	2138/20/A	Non-keratinizing SCC	High
12	Iramma Pujari	80yrs/F	9278	1803/20/B	Non-keratinizing SCC	Low
13	Nijamma	60yrs/F	145501	2445/20	Moderately differentiated endocervical adenocarcinoma	Undetected
14	Sunitha	45yrs/F	12104	292/21/A	SCC- Basaloid variant	Moderate
15	Vimala	62yrs/F	24066	1954/21	Moderately differentiated SCC	Moderate
16	Shankrawwa	64yrs/F	144326	2455/20	Small cell non-keratinizing scc	Low
17	Shanta Bai	63yrs/F	2827	847/20/A	Moderately differentiated non-keratinizing SCC	Low
18	Padmavati Bhimappa	71yrs/F	31273	6747/19	Non-keratinizing SCC	Low

19	Ramabai	75yrs/F	400065	7486/19	Non-keratinizing SCC	Moderate
20	Neelawwa	70yrs/F	40787	8040/19/C	Moderately differentiated non-keratinizing SCC	Moderate
21	Nagamma	78yrs/F	42021	8136/19	Moderately differentiated non-keratinizing SCC	Undetected
22	Neelabai	66yrs/F	30839	2975/20	Keratinizing SCC	Undetected
23	Basamma	38yrs/F	91454	3081/20	Moderate dysplasia (CIN 2)	Undetected
24	Aneesa	54yrs/F	1056	3563/20	Mild dysplasia (CIN 1)	Undetected
25	Boramma	38yrs/F	24552	3919/20	Severe dysplasia (CIN 3)	Moderate
26	Laxmi Bai	46yrs/F	85287	2489/21	Moderately differentiated SCC	Moderate
27	Shankaramma Biradar	84yrs/F	90594	2555/21	Keratinizing SCC	Undetected
28	Padmavat	49yrs/F	45070	3621/21	Mild dysplasia (CIN 1)	Undetected
29	Sarita	55yrs/F	47435	3937/21	Mild dysplasia (CIN 1)	Undetected
30	Bharati	59yrs/F	93354	965/21	Moderate dysplasia (CIN 2)	Low
31	Deepa	40yrs/F	133057	1325/21/A	Mild dysplasia (CIN 1)	Undetected
32	Shivamma	35yrs/F	62017	237/22	SCC- Basaloid variant	Low
33	Ayesha Patil	40yrs/F	161516	2935/20,	Chronic cervicitis with mild dysplasia (CIN 1)	Low
34	Roopa Hiremath	22yrs/F	40709	2246/21	Moderately differentiated non-keratinizing SCC	Moderate
35	Bhagrati	62yrs/F	176116	3683/22/A	Keratinizing SCC	High
36	Premalatha	30yrs/F	212337	3975/22	Moderate dysplasia (CIN 2)	Low
37	Shobha	52yrs/F	221112	4129/22	Chronic cervicitis with mild dysplasia (CIN 1)	Low
38	Shridevi A Rathod	27yrs/F	212016	3942/22	Mild dysplasia (CIN 1)	Undetected
39	Ambranmu	85yrs/F	67536	1210/22/A	Keratinizing SCC, Parametrium - free of tumor tissue	High

40	Yallawwa	60yrs/F	79236	3319/22	Moderately differentiated SCC	Low
41	Neelamma	50yrs/F	73489	2322/22	Invasve SCC	High
42	Shantamma	48yrs/F	78542	3197/22	Moderately differentiated SCC	High
43	Madasai	50yrs/F	69276	1535/22	Invasve SCC	High
44	Girija Deshmukh	76yrs/F	73933	2363/22	Moderately differentiated SCC	High
45	Gurudevi	42yrs/F	166971	3570/22	Keratinizing SCC	High
46	Bouramma Vittal	61yrs/F	36313	590/22	Moderately differentiated SCC	High
47	Meenakshi N	50yrs/F	102489	1086/22	Invasve SCC	High
48	Shreedevi Awadi	48yrs/F	98575	6435/22	Poorly differentiated SCC	High
49	Geeta	51yrs/F	9921	4886/22	Invasve SCC	Moderate
50	Kasturi Baddenava	45yrs/F	99209	6520/22	Moderately differentiated keratinizing SCC	High
51	Shrreedevi	34yrs/F	221152	4128/22	Papillary endocervicitis with mild dysplasia (CIN 1)	Low
52	Savita	49yrs/F	4522	3997/22	Moderately differentiated SCC	High
53	Surekha	60yrs/F	4045	8186/19	Moderately differentiated keratinizing SCC	Moderate
54	Shobha Kalagond	90yrs/F	354856	6789/22	Non-keratinizing SCC	High
55	Shilpa	46yrs/F	8901	9098/21/B	Moderately differentiated non-keratinizing SCC	Moderate
56	Sneha	46yrs/F	99045	2488/21	Moderately differentiated SCC	High
57	Rajeshwari Sulibhavi	40yrs/F	out10203	874/C	SCC- Cervical biopsy	High
58	Vijayalaxmi	52yrs/F	4567	499/20	Moderately differentiated non-keratinizing SCC	High
59	Lakkawwa Madar	48yrs/F	out30201	3136/A	SCC- Cervical biopsy	High
60	Shantamma	52yrs/F	10123	3934/21	Mild dysplasia (CIN 1)	Undetected

61	Mahadevi	55yrs/F	45550	5977/22	Severe dysplasia (CIN 3)	Moderate
62	Sumitra	62yrs/F	2345	8086/20	Moderately differentiated keratinizing SCC	High

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