Original Research Article

A Prospective Study on Expression of Human Leukocyte Antigen 1 in Cancer Cervix Patients

R. Padmavathi[1], R. Kousalya[2], C. Ezhilarasi[3]

[1]Assistant professor, Department of Pathology, Government Mohan Kumaramangalam Medical College, Salem

[2]Assistant professor, Department of Pathology, Government Mohan Kumaramangalam Medical College, Salem

[3]Assistant professor, Department of Pathology, Government Mohan Kumaramangalam Medical College, Salem

Corresponding author: Dr. R. Padmavathy

Conflict of interest: Nil

Abstract:

Introduction: Cervix is the most common site in the female genital tract which is exposed to viral and bacterial infections. Also it is the targetfor carcinogenic agents leading to invasive cancer. The local tissue immune status may play a role in cancer cervix. Routine cytological PAP smear screening, early diagnosis and curative treatment had reduced the mortality of cancer cervix. The aim of our study is to examine the expression of HLA 1 in various types of carcinoma cervix and to correlate them according to their grades of malignancy.

Methods: We chose the hysterectomy and small cervical biopsy tissues of cervical cancer and then detected the expression of HLA 1 using SP immunohistochemistry. The association of the HLA 1 expression with the clinicopathologic profiles of the patients was analyzed.

Results: The positive staining of HLA class 1 antigen in well differentiated carcinoma is 7 out of 24 SCC cases and the negative staining of HLA class 1 in poorly differentiated form of SCC is 5 out of 24 cases.

Conclusion: The expression of HLA 1 are down regulated in poorly differentiated carcinomas and it is more efficiently expressed in well differentiated forms which indicates that there is a good prognosis in well differentiated carcinomas because of HLA 1expression.

Keywords: HLA Class 1 Antigen; Cervical Cancer; Down Regulation.

Introduction

Cervix is the most common site in the female genital tract which is exposed to viral and bacterial infections. Also it is the target for carcinogenic agents leading to invasive cancer. Cervical carcinoma is the second most common malignancy in women according to the worldwide cancer statistics [1]. Patients with invasive cancer cervix have an average age of 45 years [2]. Every year,almost 50% of the reported new cases every year have been proved to be fatal. The tumor occurs most often in the older age groups but also occurs, with relatively increased frequency in young females [3]. The decline in the rate of cancer cervix is due to the effective screening programmes via papanicolaou smear [2].

Routine cytological PAP smear screening, early diagnosis and curative treatment had reduced the mortality of cancer cervix. All the features which are of diagnostic and prognostic significance cannot be revealed by routine histological techniques. 80% of the cases are of squamous cell carcinoma subtype. The next most common is Adenocarcinoma constituting 15%. Remaining 5% of the cases are contributed by Adenosquamous and Neuroendocrine carcinomas. Most of the histological subtypes are associated with high risk Human Papilloma viruses [3].

Studies have shown that there is a crucial role for high risk Human papillomaviruses in the etiology of carcinoma cervix [4]. Host immune response factors contribute to the persistent HPV infection and progression to cervical neoplasia (Cervical Intraepithelial Neoplasia and Invasive cancer). Genes in the Human Leukocyte regionof chromosome 6 are associated increased susceptibility to transforming properties of high-risk HPV. Cancer cells have many mechanisms to escape from immune-mediated recognition and destruction. Loss of cell surface expression of

Human Leukocyte Antigen (HLA) class I molecules is very important particularly, as this enables cancer cells to evade recognition and lysis by cytotoxic T lymphocytes [5].

Present study intends to analyze the clinical features and histomorphology of cancer cervix also to assess the expression of HLA I in the differentiated forms of malignant lesions of the cervix. Also to correlate the grade of malignancy and HLA 1 expression in carcinoma cervix.

Materials and Methods

This study was done as a prospective study in a tertiary care teaching hospital for a period of one year. A total number of 30 cases were included in our study. From case records brief clinical data were collected, which included age, clinical diagnosis and surgical procedure.

Hysterectomy and small cervical biopsy specimens with cervical cancer. From patients in all age groups (20 - 60 years) of abnormal uterine bleeding with suspected carcinoma cervix. VIA / VILI positive cases were included in the study. Whereas inadequate specimens, Chronic cervicitis. Squamous Intraepithelial Lesion, treated patients before surgery and specimen not sent in formalin were excluded. Among the total cases received in the Department of Pathology in our hospital during the study period, 30 cases were taken into study as per inclusion criteria and further evaluated.

All those 30 small biopsies and hysterectomy specimens are selected, then fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin. Immunohistochemistry method was done as a two-step indirect technique by which antigens were detected in cells and tissues. The first was the binding to specific epitopes of the primary antibody. Second was a calorimetric reaction to detect the bindings. Interpretation of IHC for HLA – 1, Positive staining refers to cytoplasmic or cytoplasmic membrane staining of cells [16]. Intensity of positive staining is graded between 0 to 3+.

Table 1: Interpretation of HLA -1 positivity

Sl.No	Grading	Percentage of cells expressing HLA - 1 positivity
1.	0 (negative)	No positively staining cells
2.	1+ (focally positive)	< 25 % of positively staining cells
3.	2+ (positive)	25 - 50 % of positively staining cells
4.	3+ (diffusely positive)	> 50 % of positively staining cells

Results

Of 2542 gynaecological specimens received at Pathology Department Coimbatore Medical College Hospital during the period of one year, malignancy was reported in 215 cases (8.45 %).Among which cancer cervix was 30 cases. This prospective study included routinely processed biopsies from 30 patients with malignant lesions of the cervix diagnosed histologically.

The patients diagnosed as cervical cancer were divided based on age and There was increased incidence of cervical cancer observed in the age group of 41- 50 years (40 %) followed by 51-60 years (26.7%) and 61 - 70 years (26.7 %).

We next analyzed the colposcopic findings, It includes ViA / ViLI (Visual inspection with Acetic acid and visual inspection with Lugol ' s Iodine), clinically obvious growth, cervical ulceration and erosion cases. Most common clinical finding was growth (46.7%).

Coming to the type of carcinomas diagnosed histologically, Squamous cell carcinoma is the most common type constituting 80% followed by Adenosquamous and Adenocarcinoma.

Table 2: Type of carcinoma cervix cases

Type	No. of Cases	Percentage
SCC- Well differentiated	9	30%
SCC- Moderately differentiated	8	26.7%
SCC- Poorly differentiated	7	23.3%
Adenocarcinoma	2	6.7%
Adenosquamous carcinoma	3	10%
Clear cell Ca	1	3.3%
TOTAL	30	100%

Coming to histopathologic features, large cell type was seen in 11 specimens, small cell type in 13 specimens. Keratinizing type in 10 and non- keratinizing in 14 specimens. Keratin pearls was seen in 9 specimens. Features like desmoplasia, inflammation and necrosis was also seen in few

specimens. In few specimens glandular pattern, hobnail cells were seen. Among our study population, particularly squamous cell carcinoma 9 was well differentiated, 8 were moderately differentiated and 7 were poorly differentiated. We next correlated grading of HLA 1 with type of SCC and which showed that higher the grade, carcinoma is well differentiated. Grade 1 is considered negative and grade 2 and 3 are considered positive for HLA 1.

Table 3: Expression of Hla - 1 in Squamous Cell Carcinoma

Type	1+ (< 25%)	2 + (25 -50%)	3 + (>50%)
SCC -Well Diff	2	2	5
SCC - Mod Diff	4	2	2
SCC -Poorly Diff	5	1	1

According to this, the positive expression of HLA 1 is seen in 9 out of 30 cases which are of well differentiated grade. It is negative in 7 out of 30 cases which are of poorly differentiated grade. It is positive in 4 out of 8 moderately differentiated grade of Squamous cell carcinoma. Similarly in other types of carcinoma cervix positive HLA expression was seen in 1 specimen of adenocarcinoma and one specimen of adenosquamous carcinoma.
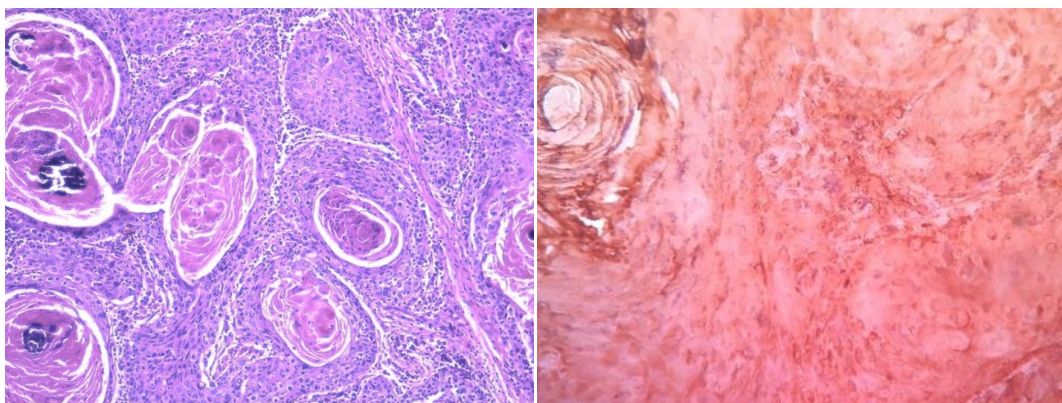

Figure 1: H&E and IHC expression –HLA-1- well differentiated squamous cellcarcinoma

Discussion

Carcinoma cervix is the second most common cancer among the world6 with increased mortality in around half of the new cases diagnosed every year. The risk of cancer cervix increases with increase in age. Because of the high prevalence of HPV infection around 25 years of age, the carcinoma incidence increases with age. Due to the persistent HPV infection, the risk of developing carcinoma increases with age 7. It constitutes about 20% of cancer cervix at the age of > 65 years.

In the present study, the youngest age group at which carcinoma cervix was reported is 39 years. Its incidence is common between 40to 50 years. Data from the cancer registries indicate that > 75% of cervical carcinomas develops in women more than 35 years of age.

Epidemiological studies have been suggested that high risk HPV infection as the primary risk factor for cancer cervix [8]. It is well established that persisting infections were the most significant risk factor for cancer cervix [9]. Almost all cervical cancers contain the genes of most common types 16, 18, 31 and 45 which are high risk groups. HPV 16 was found to be associated with tumors expressing HLA 1 antigen 10. Enormous lines of evidence suggest that cell - mediated immune responses are very important in controlling established HPV infections and HPV associated neoplasm's, especially in tumor cell clearance [11].

Being an important component of immune system, HLA system is able to present antigenic peptides to antigen - specific T lymphocytes [12]; they then trigger the immune response to eliminate tumor specific proteins.

The main function of HLA 1 is to combine with antigen and forming HLA -antigen complex and presenting them to the cell surface [13]. These cytotoxic cells can directly kill target cells via secreting substances like perforin or by inducing target cell apoptosis through Fas / Fas L way resulting in a special lethality for target cells. Hence any modification in cellular antigen - presenting system can correlate with the escape of CIN and carcinoma in situ from immunological control and then progress to invasive carcinoma .

Immune - surveillance escape mechanisms emerges as an important step in the progression of HPV associated tumors [14]. Low HLA 1 expression cannot present antigenic peptides to the

cytotoxic cells [15]. The tumor cells can avoid the specific killing effects of CTL cells, continue to progress and metastasize. Strong tumor immunity is present in patients without lymph node metastasis our Immunohistochemical study demonstrated that HLA 1 is statistically low in poorly differentiated carcinomas. The low expression of HLA 1 in cervical cancer tissue may indicate that tumor cells exhibit HLA-1downregulation, resultant loss of antigen presentation capability and down regulation of endogenous antigen processing machinery for MHC presentation. Jiang Tao Fan, Yan Liao et al[16] evaluated the expression ofHLA - 1, CD 8 and CD 4 and their clinical significance in cervical cancer. They observed that the local expression of HLA - 1, CD 8 and CD 4 in cervical tissue may be involved in the occurence and progression of cervical carcinoma. The present study also observed the expression of HLA - 1 in the cervical cancer tissue.

Ekaterina S. Jordanova et al [17] studied Human Leukocyte antigen class 1, MHC class I chain - related molecule A, and CD 8 + / Regulatory T cell ratio: which variable determines survival of cervical cancer patients. They have concluded that weak HLA-A-MICA expression combined with low CD 8 + / Treg ratio reveals a patient group with the poorest survival in cervical cancer. The present study shows that the expression of HLA - 1 is low in poorly differentiated carcinoma cervix.

Vivian M Spaans et al [18] evaluated HLA - E expression in cervical adenocarcinoma: association with improved long - term survival. They have concluded that high expression of HLA - E occurred in the majority of all histopathological subtypes of cancer cervix; especially in cervical adenocarcinomas. High HLA - E expression in cervical adenocarcinoma was associated with improved patient survival. The present study also demonstrated that HLA - 1 is expressed in all histopathological subtypes and it is more intensely expressed in well differentiated carcinomas.

Debbie M Ferns et al [19] evaluated the Classical and non - classical HLA class I aberrations in primary cervical squamous and adenocarcinomas and paired lymph node metastases. They have concluded that the tumor immune escape variants lead to metastasis. Moreover, SCC tumors shows downregulation of HLA - 1 A or total classical HLA in combination with HLA - G expression had poor prognosis. HLA expression is used as a biomarker for patient selection for CTL - and NK - cell based immunotherapeuticintervention.

Louise A. Koopman et al [20] studied about Human LeukocyteAntigen class I gene mutations in cervical cancer. In that they have concluded the nature of nucleotide insertions and single - base substitutions responsible for the complete absence of HLA class I molecules in cervical cancer in vitro and ex vivo. The present study also states that there is complete loss of expression of HLA - 1 in poorly differentiated cervical carcinoma.

Marloes Gooden, Magit Lampen et al [21] studied HLA - E expression by gynecological cancers restrains tumor - infiltrating CD 8 + T lymphocytes. They have concluded that HLA - E expression in ovarian and cervical cancer is the result of a smoldering inflammatory response. This emerging concept entails the presence of an inflammatory milieu that can either promote tumor progression or antitumor activity. The inhibiting impact of HLA - E in cervical cancer is limited, due to beneficial signs of inflammation such as high CTL infiltrate, strong viral antigens and stimulating HLA ligands.

Conclusion

The normal squamous cell expresses HLA 1 in the nucleus. The normal cervix expresses MHC class 1 antigen on the lower one third to one half of squamous epithelium. As the cell becomes neoplastic, the antigen moves from the nucleus to the cytoplasm and then to the cytoplasmic membrane. In the present study, well differentiated squamous cell carcinoma recapitulates partly the normal expression of HLA 1. As the grade of malignancy increases, the expression of HLA 1 is lost in the cytoplasm. Thus it explains that the antigen HLA 1 is expressed in well differentiated squamous cell carcinoma which chemotactically attracts CD8 + lymphocytes. In parallel, in poorly differentiated squamous cell carcinoma, HLA 1 expression was low

Understanding the above pathogenic mechanism could influence our outlook towards targeted therapy for Squamous cell carcinoma. Partly IHC of HLA 1 could help in accurate categorization of well differentiated and poorly differentiated squamous cell carcinoma in a more objective manner. Also individualised treatment strategies could be formulated based on patient's CD 8 and HLA 1 expression.

References

1. Christopher D. M. Fletcher Diagnostic Histopathology oftumors 4e vol 1:13;814- 37.
2. Robbins and Cotran Pathological Basis of Disease, 9 e Kumar, Abbas, Fausto, Aster: 2014;23: 1001- 07
3. Pathology of Female Genital tract- Blausteins

by Robert J Kurman - 5th edition.

4. M J Arends et al. Aetiology, pathogenesis, and pathology of cervical neoplasia. Clin Pathol 1998; 51: 96 - 103.

5. Aptsiauri N, Cabrera T, Mendez R, Garcia-Lora A, Ruiz-Cabello F, Garrido F. Role of altered expression of HLA class I molecules in cancer progression. 2007.

6. Guimaraes MC, Soares CP, Donadi EA, Derchain SF, Andrade LA,Silva TG, et al. Low expression of human histocompatibility soluble leukocyte antigen-G (HLA-G5) in invasive cervical cancer with and without metastasis, associated with papillomavirus (HPV). J Histochem Cytochem. 2010; 58:405–11.

7. Santin A, Bellone S, Palmieri M, et al. Induction of tumor-specific cytotoxicity in tumor infiltrating lymphocytes by HPV16 and HPV18 E7-pulsed autologous dendritic cells in patients with cancer of the uterine cervix. Gynecol Oncol 2003; 89:271-80.

8. Bontkes H, de Gruijl T, Walboomers J, et al. Assessment of cytotoxic T- lymphocyte phenotype using the specific markers granzyme B and TIA-1 in cervical neoplastic lesions. Br J Cancer 1997; 76:1352-60.

9. Chan D, Cheung T, Tam A, et al. Risk association between human leukocyte antigen-A allele and high-risk human papillomavirus infection for cervical neoplasia in Chinese women. J Infect Dis. 2005; 192:1749-56.

10. Torres LM, Cabrera T, Concha A, Oliva MR, Ruiz-Cabello F, Garrido F. HLA class I expression and HPV-16 sequences in premalignant and malignant lesions of the cervix. Tissue Antigens.1993; 41:65–71.

11. Connor ME, Stern PL. Loss of MHC class-I expression in cervical carcinomas. Int J Cancer. 1990; 46:1029–34.

12. Brady CS, Bartholomew JS, Burt DJ, Duggan-Keen MF, Glenville S, Telford N, et al. Multiple mechanisms underlie HLA dysregulation in cervical cancer. Tissue Antigens. 2000; 55:401–11.

13. Cordon-Cardo C, Fuks Z, Drobnjak M, Moreno C, Eisenbach L, Feldman M. Expression of HLA-A, B, C antigens on primary and metastatic tumor cell populations of human carcinomas. Cancer Res. 1991; 51:6372–80.

14. Akobayashi et al.Evolving Immunosuppressive microenvironment during human cervical carcinogenesis. 2008; 33: 1: 5.

15. Gonçalves MA, et al. Classical and non-classical HLA molecules and p16 (INK4a) expression in precursors lesions and invasive cervical cancer. Eur J Obstet Gynecol Reprod Biol. 2008; 141:70

16. Jiang Tao Fan, Yan Liao, Xiao Hui Si, Xiao Li Geng, Wei Wei, Qing Li Xie, Expression of HLA-I, CD 8 and CD 4 and their clinical significance in Cervical cancer: 2011; 2(1):10-15.

17. Ekaterina S. Jordanova et al. HLA - E expression by gynaecological cancers restrains tumor infiltrating CD 8+ T lymphocytes. 2011; 26: 10656- 61.

18. Spaans VM, Peters AA, Fleuren GJ, Jordanova ES. HLA-E expression in cervical adenocarcinomas: association with improved long-term survival. J Transl Med. 2012 Sep 4; 10:184.

19. Debbie M. Ferns et al. Classical and non-classical HLA class I aberrations in primary cervical squamous and adenocarcinomas and paired lymph node (2016)4:78; 1-11.

20. Louise A. Koopman, Arno R. Van der Slik et al. Human Leukocyte Antigen class 1 gene mutations in Cervical Cancer 1999; 19: 1669-77.

21. Marloes Gooden, Margit Lampen, et al. HLA - E expression by gynaecological cancers restrains tumor infiltrating CD 8+ Tlymphocytes. 2011;26: 10656- 61.