ERJ Express. Published on September 27, 2006 as doi: 10.1183/09031936.00009806

EXPRESSION OF HISTOBLOOD GROUP ANTIGENS IN BRONCHIAL SQUAMOUS METAPLASIA.

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ABSTRACT

The aim of this study is to evaluate the expression of blood group antigens in squamous bronchial metaplasia to determine if it could identify patients at risk for lung cancer.

Material and Methods: 100 bronchial biopsies were included in this study. The cases were classified according to the WHO grading system. Immunohistochemical stains for histoblood group A, B, p53 and Ki67 were performed.

Results: 56 patients (56%) belong to blood A group. Among them 6 patients (10.7%) did not express antigen in squamous metaplasia. Three of them showed carcinoma at the moment of the biopsy and the other three developed synchronous lung carcinoma. 9 patients (9%) belong to B blood group. Loss of antigenic expression was observed in 5 cases. All of them developed synchronous lung carcinoma. The patients with low-grade dysplasia and high-grade dysplasia developed lung cancer in 71% and 100% respectively.

Conclusions: Our findings suggest that the loss of histoblood antigens expression is a event in carcinogenesis of bronchial mucosa and is usually associated to high grade lesions and hyperproliferative activity

Keywords: Squamous metaplasia, squamous dysplasia, histoblood group.

INTRODUCCION

Lung carcinogenesis is a multiple steps process characterized by accumulation of successive molecular genetic and epigenetic abnormalities, resulting in epithelial cell malignant transformation [1, 2], such as expression of oncogenes, and loss of tumor-supressor genes [2, 3]. Increasing information is currently available on the genetic alterations leading the final invasive stage of bronchial carcinomas. However the natural history of the prinvasive lesions is poorly understood. Actually it´s clear that the high grade preinvasive lesions will develop into invasive carcinoma in 30-50% of the cases, whereas the majority of low grade preinvasive lesions remain stable or regressed during follow up [4, 5]. Nevertheless the efforts to better characterize lesions are need.

Histoblood group antigens are a group of glycoproteins and glycolipids whose antigenic specificity is determined by a variation in their constituent carbohydrate chains [6]. These antigens are involved in various biological process, such as cellular differentiation, maturation, proliferation, malignant transformation and intercellular signalling [7]. Prognostic significance of a loss of or modified ABH antigen expression has been suggested in carcinomas of various sites, including the urinary bladder [8], gastrointestinal tract [9] and lung [10-12]. Davidsohn and Ni [10] demonstrated a loss of ABH determinants in pulmonary carcinomas. Subsequently Lee JS et al [11] and Moldvay et al [13] demonstrated that a reduction or deletion of antigenic expression correlated with a reduced survival rate. Matsumoto et al [14] concluded that this loss of ABH blood group antigens in lung carcinomas correlated with their metastatic potential, especially with recurrence and hematogenous metastasis. Similar finding were reported by Ichikawa et al [15].

The aim of this study was to explore the significance of the expression of histoblood antigens in squamous metaplasia and its association to other prognostic

factors such as oncoprotein p53 expression and cellular proliferation index (Ki67) by immunohistochemical techniques.

MATERIAL AND METHODS

The study material were obtained from the files of the Department of Pathology of the Gregorio Marañon Hospital and consisted of bronchial biopsies specimens obtained during bronchoscopy from 100 patients with respiratory symptoms and clinical suspicion of lung cancer, between 1996–2000. The lesions were classified and graded according to the World Health Organization (WHO) classification [16].

Demographic information was obtained from clinical records. Smokers were defined as patients who smoked at the time of biopsy. Ex-smokers were defined as those who had quit smoking more than one year before the time of biopsy and no smoker those patients who denied the habit. Tobacco consumption was evaluated as a pack/year index ([number of cigarettes smoked per day x number of years of smoking]/20). In the follow-up synchronous carcinoma was defined as a carcinoma diagnosed within a year after a biopsy demonstrating squamous metaplasia. Metachronous carcinoma was defined as occurring at least 1 year afterward.

Preparation of Tissues: Tissues were fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin.

Immunohistochemical Studies: Sections 4 μm thick from paraffin-embedded biopsy specimens were cut and mounted on glass slides. The specimens were deparaffinized in xylene and rehydrated step by step with descending concentrations of ethanol. Sections were incubate at 37ºC with 0.3% H_2O_2 in absolute methanol for 10 min, to block endogenous peroxidase. After being washed with phosphate-buffered saline (PBS), for pH 7.2 for 20 min and incubated with the primary antibodies for 45

min in a moist chamber at room temperature. Primary antibodies used were: anti-blood group A (Clon: T36, from Signet Laboratories, Dedham, MASS 1:60 dilution)), anti-blood group B (Clon: CLCP-19B, from Signet Laboratories, Dedham, MASS 1:60 dilution), anti-p53 (Clon: DO7, from Novocastra, 1:100 dilution) and the antibody against the Ki67 (Clon: MIB-1, from DAKO Corporation, California, 1:200 dilution) after pre-treated in a microwave oven (15 min, 500 W) in citrate buffer (10 mM, pH 6.0)

Sections were subsequently incubated with biotinylated anti-mouse and anti-rabbit Ig G and LBA (from DAKO) for 25 min at room temperature, rinsed in PBS for 5 min, and immersed in avidin peroxidase complex for 25 min. Finally the peroxidase was localised by treatment of the samples with a fresh mixture of diaminobenzidine and substrate in 10 min. After washing in distilled water, the sections were lightly counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene and coverslipped using permount.

Immunostaining to A and B anti-groups was defined as positive when the cells showed a granular staining in cytoplasm and/or cytoplasmic membrane. Regardless it's intensity. Inmunohistochemical reactivity to p53 and Ki67 was recorded as the percentage of stained nuclei; we deemed them positive when more than 10% of the nuclei were stained. The intensity was divided into five groups by percentage of stained cells as follows: 0 = 0-10%, + = 10-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%.

Statistics Analysis: Data analysis was made by SPSS package 11.5 version. The p value was calculated by χ^2 according to Yates´ Test and finally corrected by the exact Fisher´test if any of the values were <5. The association of variable strength was made

as well as the possible risk to develop any respiratory neoplasia by Odds Ratio and 95% confidence intervals.

RESULTS:

The characteristics of the 100 patients are shown in Table 1. Bronchial lesions were graded according to WHO grading system. One case showing Angiogenic Squamous Dysplasia (ASD) was classified as severe dysplasia. All these patients had respiratory symptoms related with lung cancer but not yet lung cancer diagnosis.

56 patients belong to blood A group (56%). All of them showed a positive staining for histoblood group in endothelium and red blood cells in the biopsy sample. Among them six patients (10.7%), showed no expression of histoblood A antigen in bronchial epithelium. One case showed mild dysplasia (16.6%), two cases showed severe dysplasia (33.3%), two cases showed in situ carcinoma (33.3%), and one case carcinoma microinvasive (16.6%). Three patients (3/6) 50% showed carcinoma at moment of the biopsy. All the other three were diagnosed of lung carcinoma along the first year after biopsy (synchronous). All cases with loss of expression of the A histoblood group showed positivity for Ki67 and p53, according to previously described criteria. High intensity Ki67 stained cells were located in the basal and intermediate levels of metaplastic epithelium with a mean of 70% stained cell. Similar results were observed for p53 protein with an 85% stained cells. The intensity of staining was very strong. The loss of A antigen show a significant relationship to Ki67 positive staining ($p = 0.03$) (Table 2). Among the remaining cases without loss of expression of A histoblood group, 22 patients (39.3%) developed synchronous lung carcinoma, 7 cases (12.5%) developed metachronous lung carcinoma, 10 cases (17.9%) did not developed neoplasia and 11 cases (19.6%) were lost for follow-up**.** The case with ASD was

associated loss group A histoblood antigen expression, and showed a positive stain to oncoprotein p53 and Ki67 and developed synchronous lung carcinoma *(Figure 1)*.

Nine patients belonged to B blood group. Loss of antigenic expression was observed in five cases (55%). Four cases showed moderate dysplasia and one case mild dysplasia. All five cases (100%) with loss of antigenic expression developed synchronous lung carcinoma. p53 and Ki67 staining was positive en all cases (Table 3). Concerning the four cases without loss of expression of B histoblood group (45%), three cases (33.3%) developed metachronous lung carcinoma, and one case (11.1%) was lost for follow-up.

The predictive values of the loss histoblood A and B groups antigens in preinvasive bronchial lesions to the development of synchronouos and metachronous lung carcinoma the results were: positive predictive value = 24% and the negative predictive value = 100%.

The follow up was available for 82 cases. The mean follow up was 24.7 (range 6-48) months. 68.3% (56/82) cases develop synchronous carcinomas. Squamous lung cancer was the most frequent histologycal type in 28% of the cases (23/82). Small cell lung carcinoma developed in 6.1% of the cases (5/82). Lung adenocarcinoma in 3.7% of the cases (3/82) and 8.5% of the cases (7/82) disclosed carcinoma in other localizations, six cases (7.3%) laryngeal carcinoma and one case breast carcinoma. 12.2% of the cases (10/82) developed metachronous carcinomas: six squamous lung carcinoma, one case lung adenocarcinoma and three cases with carcinoma in other localizations (2 cases in larynx and one ureteral). Eighteen patients (21.9%) with clinical and radiological criteria of lung cancer, died without histology diagnostic. sixteen cases (19.5%) did not develop neoplastic lesions in the 48 months of follow-up (Table 4).

After correlating the grade of dysplasia with the development of lung cancer in patients with follow-up, we found that among fifty five patients bearing low grade lesions (basal cell hyperplasia (BCH), metaplasia without dysplasia, and mild dysplasia), Thirty four patients (61.8%) developed synchronous carcinoma, five patients (9.1%) metachronous carcinoma, and sixteen patients (29.1%) did not develop cancer. 81.5% (22/27) of high grade lesions (moderate and severe dysplasia), developed synchronous carcinoma, and 18.5% (5/27) metachronous carcinoma, demonstrating a statistically significant association between high grade lesions and the development lung cancer p=0.006. Sixty three cases (63%) showed staining of more than 10% of the cell population of the plaque for Ki67 and sixty six cases (66%) to p53. When comparing Ki67 positivity and p53 with the grade of dysplasia, it becomes apparent that high grade lesions show a higher number of positive cases than low grade ones (47.8% as 77.4%) (p=0.005); (60.8% as 80.8%) (p=0.05); respectively.

DISCUSSION:

The histologic grading of squamous dysplastic changes is still used as a gold standard to address the malignant potential of those lesions [17]. However a recent study of Breuer et al [5] showed that 9% of the squamous metaplasias and low grade (mild and moderate dysplasias) progressed to carcinoma in situ and invasive carcinoma, suggesting that a stepwise histopathologic multistage development of lung carcinoma does not always occur or it is not always detected because rapid progression. They conclude that the histological grade of any preneoplastic lesion cannot be reliably used for accurate risk assessment of field carcinogenesis, because cannot differentiate the true potential malignant of preneoplastic aquamous lesions.

Although the risk of lung cancer increases with the presence of preinvasive lesions, the molecular determinants predicting the irreversible progression to lung cancer have not been identified.

The demonstration of variations in the immunohistochemical profile of expression of A and B histoblood groups antigens, similar to that seen carcinoma, would be of clinical significance not only to prognosticate lung carcinomas but also as a useful tool for early diagnosis. Deletion or reduction of histoblood A or B epitopes has been extensively studied in human cancer. After initial report in gastric cancer [18], it was described and correlated with histological grade and metastatic potential in other gastrointestinal [9], lung [10-12], cervical [19], oral [20] and bladder carcinoma [8]. As far as bronchial carcinoma is concerned previous reports have suggested that patients belonging to A or B blood groups and bearing tumors which do not express the corresponding histoblood groups show a shorter survival after diagnosis [13, 14, 21]. Concerning histoblood antigen expression, our results suggest that antigenic loss occurs in the cancerization process of bronchial mucosa, as it is a rare event in low grade squamous lesions but on the contrary is frequent in high grade lesions associated to developed synchronous lung carcinoma [10-12, 14, 15]. We considered that in spite of the fact that the size of our series is too short to talk about predictive or diagnostic value, the trends found in our date suggest that the presence of loss of histoblood antigen expression in preinvasive bronchial lesions could reveal a severe field cancerization process of bronchial mucosa and this can be a feature shown by preinvasive bronchial lesions associated to synchronous carcinoma. It must be emphasized that the study included patients with clinical suspicion of lung cancer.

Data presented in this study showed a high percentage (68.3%) of synchronous cancer associated to preinvasive bronchial lesions. This fact can be explained by two

different hypotheses on the one hand it could support the theory that the sequential stepwise histological progression of preinvainvasive bronchial lesions does not always occurs [5]. On the other hand it may reflect that field cancerization may affect randomly any site in the bronchial tree. So concomitant lesions can be of different age and can progress toward invasion at different rates [22]. The results of our study unfortunately can not clarify this point.

The alteration of tumor supressor genes plays a critical role in the development of many forms of cancer. So we have tried to correlate this with histoblood antigen expression. The disturbance of the normal function of p53 oncoprotein has been suggested to reflect an important event during malignant transformation. Some authors have reported data showing p53 overexpression in squamous bronchial metaplasia [23] and p53 mutations associated to high grade dysplasia [1]. Other authors have studied metaplastic lesions from patients with coexisting carcinomas, showing p53 overexpression [23, 24]. The results in our study are in accordance to these data.

All cases with loss of expression of the histoblood antigen also show a high staining to Ki67 and p53 in the present series. These results confirm that the loss of antigenic expression combined with other factors such as p53 expression and increased cellular proliferation, are at least simultaneous events, rendering necessary a larger series of cases to evaluate its statistical significance. These finding support the progressive and stepwise accumulation of genetic/epigenetic abnormalities leading cell transformation [1, 2, 25, 26].

Angiogenic Squamous Dysplasia (ASD) is characterised by small capillaries growing underneath epithelium in a micropapillary pattern, indicating abnormal vascularization of the bronchial mucosa that is reflected by both increased microvascular density and aberrant morphology of the bronchial capillary bed. Possible

mechanisms of angiogenesis in ASD may involve small populations of dysplastic squamous cells harbouring premalignant mutations transmitting angiogenic signals over very short distances [27]. In our study we observed only one case with ASD. It was associated to loss of group A histoblood antigen expression, oncoprotein p53 and Ki67 positivity and developed synchronous carcinoma. This finding gives additional support to the opinion that it must be classified as a high grade lesion.

In conclusion our findings suggest that the loss of histoblood antigens expression is a event in carcinogenesis of bronchial mucosa and is usually associated to high grade lesions and hyperproliferative activity. Further studies are needed to better characterize the alterations that define the malignancy of the bronchial epithelium, as well as to identify critical factors, or association of factors that would be predictive of the progression of preinvasive bronchial lesions to invasive cancers. Additionally with very sensitive and specific methods and acceptable reproducibility to endobronchial detection of the malignant cell clones, such studies may help to develop a more effective strategy for the management of this highly fatal disease.

REFERENCES:

1. Jeanmart M, Lantuejoul S, Fievet F, Moro D, Sturm N, Brambilla C, Brambilla E. Value of immunohistochemical markers in preinvasive bronchial lesions in risk assessment of lung cancer. *Clin Cancer Res* 2003; 9: 2195-2203
2. Hirsch F R, Merrick D T, Franklin W A. Role of biomarkers for early detection of lung cancer and chemoprevention. *Eur Respir J* 2002; 19: 1151-1158
3. Thiberville L, Payne P, Vielkinds J, LeRiche J, Horsman D, Nouvet G, Palcic B, Lam S. Evidence of cumulative gene losses with progression of premalignant epithelial lesions to carcinoma of the bronchus. *Cancer Res* 1995; 55: 5133-5139
4. Risse E K, Vooijs G P, van't Hof M A. Diagnostic significance of "severe dysplasia" in sputum cytology. *Acta Cytol* 1988; 32: 629-634
5. Breuer R H, Pasic A, Smit E F, van Vliet E, Vonk Noordegraaf A, Risse E J, Postmus P E, Sutedja T G. The natural course of preneoplastic lesions in bronchial epithelium. *Clin Cancer Res* 2005; 11: 537-543
6. Watkins W M. Biochemistry and Genetics of the ABO, Lewis, and P blood group systems. *Adv Hum Genet* 1980; 10: 1-136, 379-185

7. Sell S. Cancer-associated carbohydrates identified by monoclonal antibodies. *Hum Pathol* 1990; 21: 1003-1019
8. Cuadrado E, Rodriguez-Trinidad A, Blasco E, Torrado J, Lopez Garcia J A, Arozena F. Blood group isoantigens ABO (H) in transitional carcinoma of the bladder: a clinicopathological study. *J Urol* 1986; 135: 409-415
9. Slocombe G W, Berry C L, Swettenham K V. The variability of blood group antigens in gastric carcinoma as demonstrated by the immunoperoxidase technique. *Virchows Arch A Pathol Anat Histol* 1980; 387: 289-300
10. Davidsohn I, Ni L Y. Loss of isoantigens A, B, and H in carcinoma of the lung. *Am J Pathol* 1969; 57: 307-334
11. Lee J S, Ro J Y, Sahin A A, Hong W K, Brown B W, Mountain C F, Hittelman W N. Expression of blood-group antigen A--a favorable prognostic factor in non-small-cell lung cancer. *N Engl J Med* 1991; 324: 1084-1090
12. Alvarez-Fernandez E, Carretero-Albinana L. Expression of blood group antigens by normal bronchopulmonary tissues and common forms of pulmonary carcinomas. *Arch Pathol Lab Med* 1991; 115: 42-49
13. Moldvay J, Scheid P, Wild P, Nabil K, Siat J, Borrelly J, Marie B, Farre G, Labib T, Pottier G, Sesboue R, Bronner C, Vignaud J M, Martinet Y, Martinet N. Predictive survival markers in patients with surgically resected non-small cell lung carcinoma. *Clin Cancer Res* 2000; 6: 1125-1134
14. Matsumoto H, Muramatsu H, Shimotakahara T, Yanagi M, Nishijima H, Mitani N, Baba K, Muramatsu T, Shimazu H. Correlation of expression of ABH blood group carbohydrate antigens with metastatic potential in human lung carcinomas. *Cancer* 1993; 72: 75-81
15. Ichikawa D, Handa K, Withers D A, Hakomori S. Histo-blood group A/B versus H status of human carcinoma cells as correlated with haptotactic cell motility: approach with A and B gene transfection. *Cancer Res* 1997; 57: 3092-3096
16. Travis WD, Brambilla E, Mûller-Hermelink HK, C H. WHO. Tumors of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press, 2004
17. Wistuba, II. Histologic evaluation of bronchial squamous lesions: any role in lung cancer risk assessment? *Clin Cancer Res* 2005; 11: 1358-1360
18. Oh-Uti k. Polysaccharides and a glycidamin in gastric cancer tissue. *Tohoku J. Exp. Med* 1949; 51: 297-304
19. Davidsohn I, Stejskal R. Tissue antigens A, B and H in health and disease. *Haematologia (Budap)* 1972; 6: 177-184
20. Dabelsteen E, Vedtofte P, Hakomori S, Young W W, Jr. Accumulation of a blood group antigen precursor in oral premalignant lesions. *Cancer Res* 1983; 43: 1451-1454
21. Graziano S L, Tatum A H, Gonchoroff N J, Newman N B, Kohman L J. Blood group antigen A and flow cytometric analysis in resected early-stage non-small cell lung cancer. *Clin Cancer Res* 1997; 3: 87-93
22. Lam S, MacAulay C, Hung J, LeRiche J, Profio A E, Palcic B. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope device. *J Thorac Cardiovasc Surg* 1993; 105: 1035-1040
23. Hirano T, Franzen B, Kato H, Ebihara Y, Auer G. Genesis of squamous cell lung carcinoma. Sequential changes of proliferation, DNA ploidy, and p53 expression. *Am J Pathol* 1994; 144: 296-302
24. Sundaresan V, Ganly P, Hasleton P, Rudd R, Sinha G, Bleehen N M, Rabbitts P. p53 and chromosome 3 abnormalities, characteristic of malignant lung tumours,

are detectable in preinvasive lesions of the bronchus. *Oncogene* 1992; 7: 1989-1997
25. Chyczewski L, Niklinski J, Chyczewska E, Niklinska W, Naumnik W. Morphological aspects of carcinogenesis in the lung. *Lung Cancer* 2001; 34 Suppl 2: S17-25
26. Kerr K M. Morphology and genetic of pre-invasive pulmonary disease. *Current Diagnostic Pathology* 2004; 10: 259-268
27. Keith R L, Miller Y E, Gemmill R M, Drabkin H A, Dempsey E C, Kennedy T C, Prindiville S, Franklin W A. Angiogenic squamous dysplasia in bronchi of individuals at high risk for lung cancer. *Clin Cancer Res* 2000; 6: 1616-1625

Table 1. Characteristics of 100 patients with bronchial lesions included in this study

Characteristics	Nº
Gender	
Male	92
Female	8
Mean age (y), (range)	65.5 (32-85)
Smoking History	
Current smokers	19
Ex–smokers	73
Never smokers	8
Median (range)pack/years smoked	44 (0-110)
Bronchial Lesion	
HCB	4
Metaplasia without dysplasia	42
Mild dysplasia	23
Moderate dysplasia	17
Severe dysplasia	6
CIS	6
Microinvasive Carcinoma	2

Table 2. Intensity of expression p53 and ki67 in cases with loss of expression of histoblood antigen A and histological type of carcinoma

Case	*Lesion*	*p53*	*Ki67*	*Synchronous Carcinoma*	*Histology Diagnostic*
1	Carcinoma In situ(CIS)	++++	++++	yes	Squamous Ca.
2	Carcinoma Microinvasive	++++	++	yes	Adenocarcinoma
3	Severe Displasia	++++	++++	yes	Squamous Ca.
4	Ca. In situ	++++	+++	yes	Squamous Ca.
5	Severe Displasia	+++	+	yes	Squamous Ca.
6	Mild Displasia	+++	++	yes	Squamous Ca.

(+ = 10-25%); (++ = 26-50%); (+++ = 51-75%); (++++ = 76-100%)

Table 3. Intensity of expression p53 and ki67 in cases with loss of expression of histoblood antigen B and histological type of carcinoma

Case	*Lesion*	*p53*	*Ki67*	*Synchronous Carcinoma*	*Histology Diagnostic*
1	Moderate D.	+	++	yes	Adenocarcinoma
2	Moderate D.	+	++	yes	Squamous Ca.
3	Moderate D.	++++	+++	yes	Squamous Ca.
4	Moderate D.	+++	+	yes	without histology diagnostic
5	Mild D.	+	+	yes	without histology diagnostic

(+ = 1-25%); (++ = 26-50%); (+++ = 51-75%); (++++ = 76-100%)

Table 4. Histological types of carcinomas synchronous and metachronous developed by patients of this study

Histological Type of Carcinoma	*Synchronous*	*Metachronous*	*Total*
Squamous Lung Carcinoma	*23*	*6*	*29*
Adenocarcinoma Lung	*3*	*1*	*4*
Oat Cell Lung Carcinoma	*5*	*0*	*5*
Carcinoma others localizations			
Squamous carcinoma of the Larynx	*6*	*2*	*8*
Breast Carcinoma	*1*		*1*
Ureteral Carcinoma		*1*	*1*
Without Histology Diagnostic	*18*	*0*	*18*
Total	*56*	*10*	*66*

Figure 1. **A**. Angiogenic Squamous Dysplasia h.e. 40x. **B.** Positive staining for histoblood A group in endothelium and negative in epithelium 40x. **C.** Expression p53 20x.

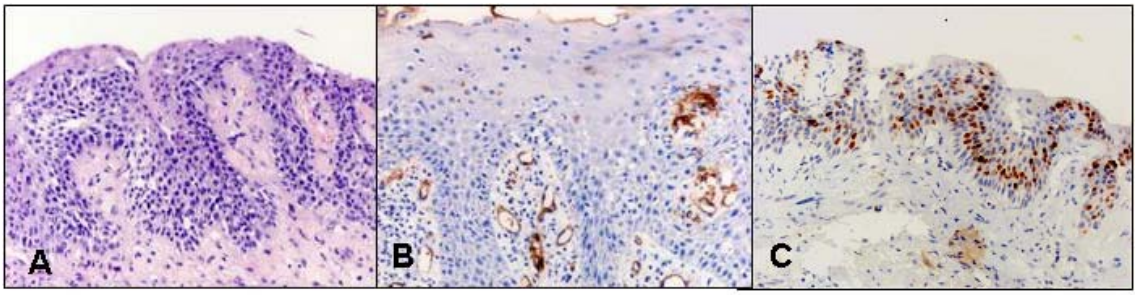

Figure 1