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Cytokeratin 18 and vimentin expression in breast cancer tissues from Sudanese patients

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ABSTRACT

Cytoskeleton proteins, including cytokeratin 18 and vimentin, are abnormally expressed in breast cancer tissues. The aim of this study is to investigate the expression of cytokeratin 18 and vimentin in cancerous breast tissues compared to normal tissues from Sudanese patients. 62 parrafin imbedded cancerous breast tissues from Sudanese patients were obtained from the Radiation and Isotope Center in Khartoum (RICK) compared to 32 normal tissues. Cytokeratin 18 and vimentin expression was investigated in the normal and the cancerous tissues using immunohistochemistry. Regarding the cytokeratin 18, the immunohistochemistry showed that 53(85.5%) of the cancerous tissues were positive and 9 (14.5%) were negative compared to two positive and 30 (93.75%) negative normal tissues. The results of vimentin showed that all the cancerous tissues were positive compared to 30 (93.75%) positive control tissues. Vimentin is not a promising tissue tumor marker while cytokeratin 18 can be considered as a useful tissue marker which can be used as a target for breast cancer therapy or in vaccination trials.

Key words: cytoskeleton proteins, breast cancer tissues, Sudanese patients

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INTRODUCTION

The cytoskeleton is a cellular skeleton, composed of proteins interacting extensively with cellular membranes and it provides the cell structure and shape [1, 2]. Cells contain three main kinds of cytoskeletal filaments, which are microfilaments, intermediate filaments, and microtubules [3].

Microfilaments (actin filaments) are the thinnest filaments of the cytoskeleton and they include actin, myosin, tropomyosin and troponin. Microfilaments play major roles in muscle contraction, cell motility, cell division and maintenance of cell junctions and cell shape [2].

Intermediate filaments are involved in the maintenance of cell shape and its three dimensional structure, anchoring of organelles and they participate in some cell-cell and cell-matrix junctions. Examples of intermediate filaments proteins are vimentins and keratins [2].

Macrotubules are polymers of alpha and beta tubulin. They participate in organelles transport and formation of mitotic spindle [2].

Cytokeratin-18
Cytokeratin- 18 is a heterotetramer cytoskeleton protein with two types; I and II. It contains 430 amino acids. It is expressed in the cytoplasm, nucleus and nucleolus of colon, placenta and liver cells and weakly expressed in the lymph nodes of breast carcinoma [4, 5]. Together with cytokeratin- 8, cytokeratin- 18 is involved in the uptake of thrombin- anti thrombin complex by liver cells, reorganization of filaments and interleukin-6 (IL-6) mediated protection [6, 7, 8]. Cytokeratin-18 is well known to be considered as one of the liver cirrhosis causing factors [9].

Vimentin
Vimentin is an intermediate filament of the cytoskeleton proteins. It is composed of two polypeptide chains with 466 amino acids [10]. It is highly expressed in the cytoplasm of fibroplasts, T and B lymphocytes and in hormone independent mammary carcinoma cell lines [11, 12]. Vimentin plays a central role in supporting and anchoring the organelles to the cytosol of the cells and it is responsible for maintaining the cell shape and the integrity of the cytoplasm [13]. It is involved in controlling the transport of Low Density Lipoprotein (LDL) and the LDL derived cholesterol through the cytoplasm of the cells [14].

Clinically vimentin is used as a sarcoma tumor marker [15]. However, increased levels of methylated vimentin have been observed in colon and upper gastrointestinal tract cancers [16].

Objective:
The objective of this article is to investigate the expression of cytokeratin 18 and vimentin in cancerous breast tissues compared to normal tissues from Sudanese patients.

MATERIALS AND METHODS

Approval and Ethical license
This study was implemented after approval from the atomic energy council of Sudan Academy of Sciences (SAS) and after obtaining an ethical license from health authorities

Study Design
This study is hospital based quantitative, non experimental, retrospective, descriptive and case control study.

Study Population
Sixty two blocks of paraffin imbedded cancerous breast tissues were involved in this study compared to 32 normal paraffin embedded blocks (out of the 62 cancerous tissues 32 were with healthy margins). The tissue samples were obtained from the Radiation and Isotope Centre in Khartoum (RICK). The histology and the grade of each cancerous tissue were determined by a well trained histpathologist.

Samples
From each block two paraffin sections were cut into 3µm thick section floated into preheated floating water bath at 40˚c, four slides were coated with adhesive salinized glass slide for Immunohistochemistry.

Technique
Immunohistochemistry technique was used to evaluate the expression of vimentin and cytokeratin 18 in the cancerous and normal breast tissues as follows: two primary monoclonal mouse anti human cytokeratin 18 (CK 18) and vimentin from DAKO with code numbers IS618 and IS630 were used respectively.

Three micrometers from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized slides (Dako). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for CK18,Vimentin using PT link. Briefly, slides were placed in Coplin jars containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at high Temp for 10minutes then allowed to cool at RT. Endogenous peroxidase activity were blocked with 3% hydrogen peroxide and methanol for 10 min, then Slides were incubated with 100-200 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then rinsed in Phosphate buffer saline. The primary antibody for CK18 and Vimentin were ready to use (Dako, Carpintera). After washing with PBS for 3 min, binding of antibodies were detected by incubation for 20 minutes with dextran labelled polymer (Dako- EnVision TM Flex kit). Finally, the sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra

hydrochloride(DAB) (Dako) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were counterstained with haematoxylin. For each run of staining, positive and negative control slides were also prepared. The positive control slides were containing the antigen under investigation and the negative control slides were prepared from the same tissue block, but were incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results were confirmed by a consultant histopathologist.

RESULTS

Description of the study population

The ages of the study group were divided to four groups ≥14, (15- 44), (45- 64) and 65≤ . However, the most affected age groups were (15- 44) and (45- 64). According to the histopathology reports the cases were classified as invasive ductal carcinoma (50), invasive lobular carcinoma (6), metaplatic carcinoma (1), mucinous carcinoma (1), papillary carcinoma (2) and ductal carcinoma insitu (2).. Regarding the grades of the breast cancer, the grade of four cases was not registered, grade 1 (5), grade 2 (19), grade 3 (34) [Table 1].

Results of the cytokeratin 18 and vimentin expression

Regarding the expression of cytokeratin18 in the tumor tissues, 53 (85.5%) of the tissues were positive while 9 (14.5%) were negative [Fig.1]. The nine negative cases were diagnosed as invasive ductal carcinoma (7, 14%), metaplastic carcinoma (1, 100%) and lobular carcinoma (1, 16.7%).

The grades of the positive tissues were grade I (5), grade II (14), grade III (31) and the grade of three tissues were not determined. The 9 negative tissues were classified as grade II (5), grade III (3) and the grade of one tissue was not determined (missing) [Fig.3].

The immunohistochemistry results of the normal tissues of cytokeratin 18 showed that 2 (6.25) of them were positive while 30 (93.75%) were negative [Fig.1 and Fig.3].

Concerning the results of vimentin expression in the tumor sections, they were all positive [Fig.2 and Fig.4]. 30 (93.75%) of the normal tissues were positive while two were negative [Fig.2 and Fig.4].

DISCUSSION

Our study showed that cytokeratin 18 was positive in 85.5% of the cancerous tissues and negative in 14.5% . This finding is comparable to the finding of Vora H H etal 2009 [17] and Enrique Lerma etal 2007 [18] who registered loss of keratin expression in 11% and 19% of breast carcinoma respectively. However, Ute Woelfe and his colleagues in 2004 [19] stated that cytokeratin 18 was down regulated in 25.4% of primary carcinoma breast tissues. Unlike our findings, Verma S and research group [20] registered that all their study population (100 breast carcinoma tissues) demonstrated positivity for cytokeratin 18.

From our results; 14% of the invasive ductal carcinoma were negative compared to 16.7% of lobular carcinoma. However, Ute Woelfe and his colleagues [19] found CK18 expression was down regulated in 17% of the lobular carcinoma cases and 25% of the ductal carcinoma specimens.

Regarding the normal tissues we found that 93.75% were negative for cytokeratin 18 while Victor E Gould and his research team [21] concluded that all the normal tissues were positive for all the cytokeratins they have studied.

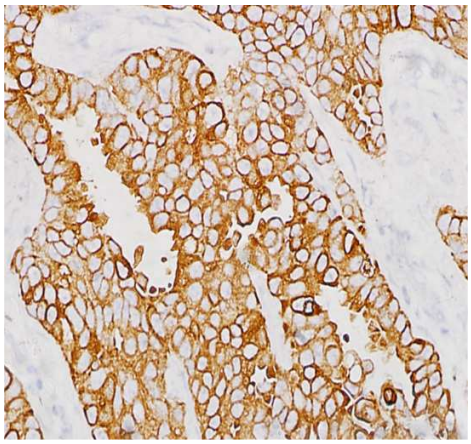
Our results showed that all the 62 cancerous tissues were positive for vimentin. The contacted previous researches did not show expression of vimentin in all the studied populations. The previous studies showed that vimentin was positive in 94% of invasive ductal carcinomas [22] and in 86% of lobular carcinoma cases [23]. Seshadri R and his team in 1996 [24] stated that 80% of breast tumor cells were vimentin positive and it was significantly associated with high tumor grade. It was registered that vimentin expression was seen in 57% of breast carcinoma patients [17], in 55% of basal like breast carcinoma cases [18], in 53% of ductal carcinoma patients [23], 37% (10 out 27) of breast carcinoma patients [21], in 32% of infiltrating ductal carcinoma patints [25], in 21.2% of invasive ductal carcinoma [26], in 19% of invasive ductal carcinoma patients [27], in 18% of breast carcinoma [28] and in 5% of lobular carcinoma cases [27].

Previously it was mentioned that vimentin was not expressed in all the lobular carcinoma samples [25] and mucinous breast carcinoma [29] while we have registered vimentin expression in all the breast cancer tissues including the lobular and mucinous breast carcinomas.

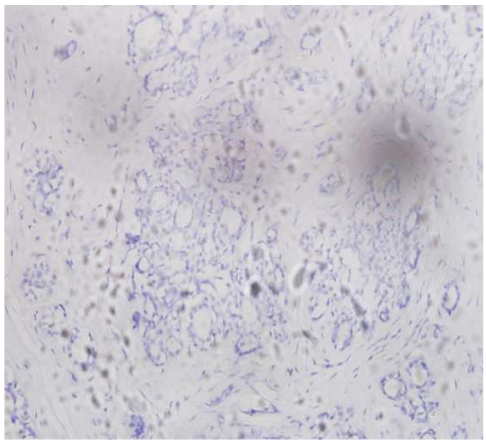
Concerning the expression of vimentin in the normal tissues, Victor E Gould and his colleagues in 1990 [21] registered expression of vimentin in 66.6% (8/12) of their study population, however we registered that 30 (93.75%) normal tissues were vimentin positive.

In 2007, Enrique Lerma and his team [18] studied cytokeratin and vimentin expression in basal like breast carcinoma tissues and they came to the conclusion that cytokeratin was positive in 81% and vimentin in 55% of their study population while Vora HH research group in 2009 [17] mentioned that cytokeratin expression was seen in 89% (absent in 11%) of breast cancer patients and vimentin expression was seen in 57% of their study population. However, we have studied the expression of vimentin and cytokeratin 18 in breast cancer tissues and we have registered that cytokeratin 18 was expressed in 85.5% of the breast cancer tissues and vimentin expression was seen in all the studied tissues.

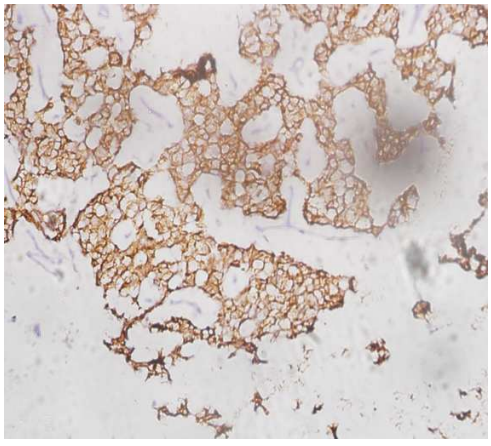
Fig 1: Cytokeratin 18 immunohistochemistry results of the cancerous and normal tissues



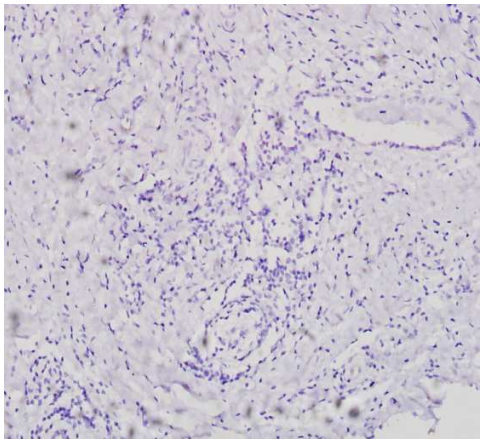
Cancerous positive x40



Cancerous negative x40



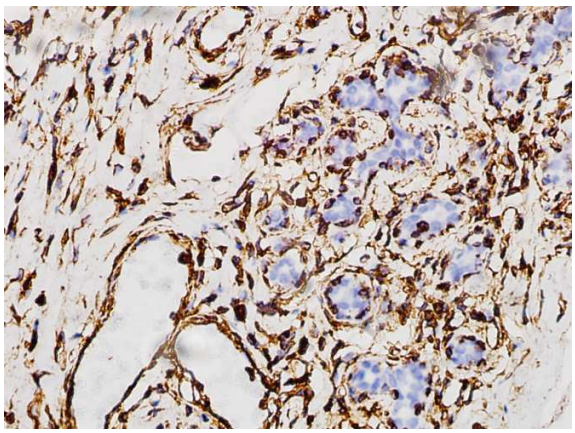
Normal positive x40



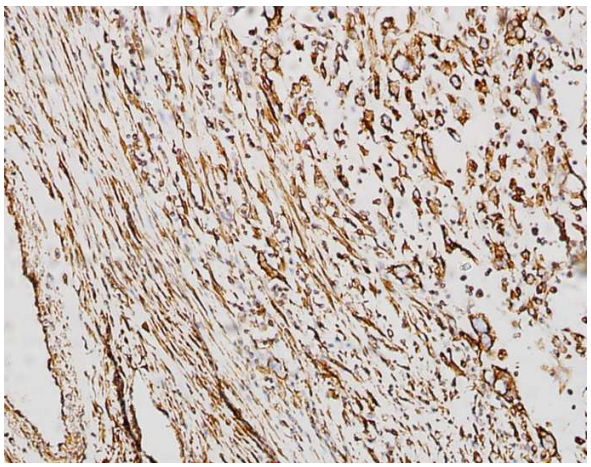
Normal positive x20

Representative samples of the cytokeratin 18 immunohistochemistry results of the normal and cancerous breast tissues.

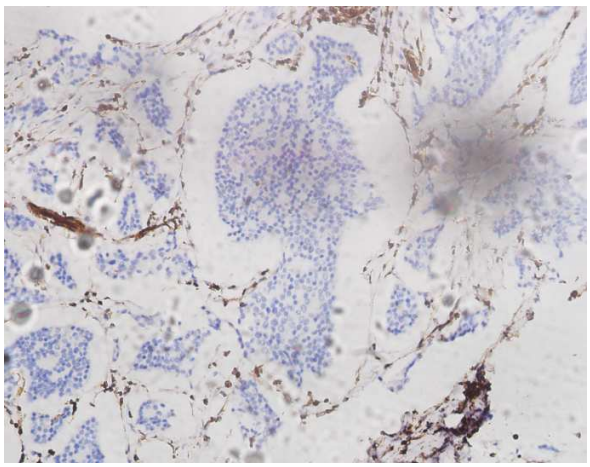
Fig 2: Vimentin expression in the cancerous and normal tissues
(Representative samples)



Cancerous positive x40



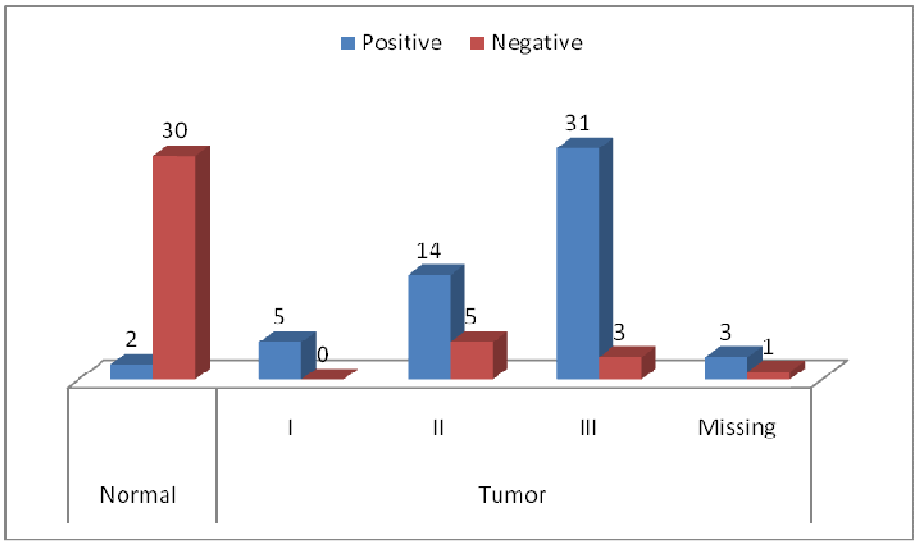
Normal positive x20



Normal negative x40

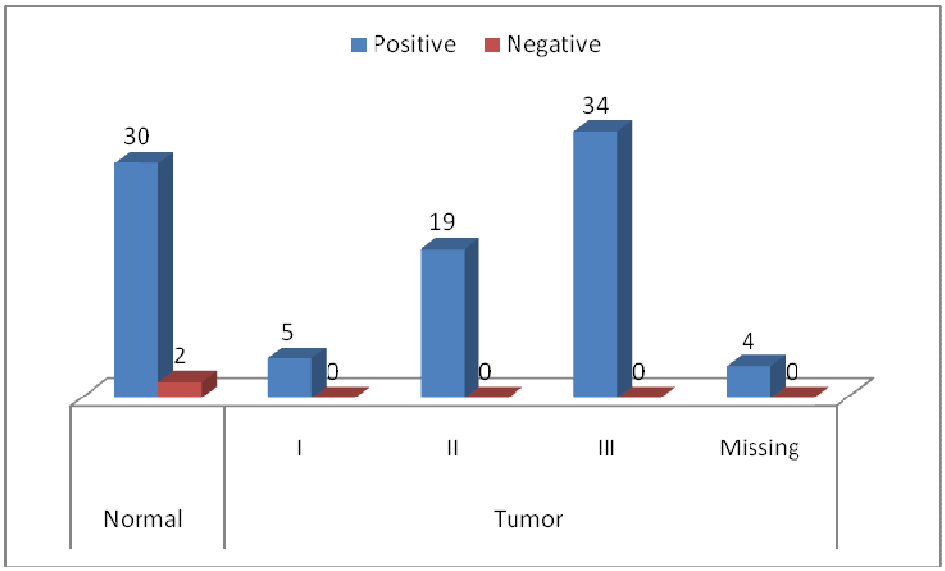
Samples showing the different vimentin immunohistochemistry results of the cancerous and normal breast tissues.

Fig.3 Cytokeratin 18 expression profile of the breast normal and tumor tissues



53 cancerous tissues expressed cytokeratin 18 and 9 were negative. The bulk of the negative tissues were classified as grade II (5/9). Concerning the normal tissues, 30 of them were negative for cytokeratin 18 and two were positive.

Fig.4 Expression of vimentin in the breast normal and tumor tissues



All the cancerous tissues were positive for vimentin compared to 30 positive and 2 negative of the normal tissues

Table 1: Descriptive information of the study population

Age group		**Breast cancer type**						**Grade**			
Range	No	Ductal	Lobular	Metaplastic	Mucinous	Papillary	In situ	I	II	III	missed
≥ 14	0	0	0	0	0	0	0	0	0	0	0
15- 44	27	22	3	1	0	1	0	3	10	13	1
45- 64	25	18	3	0	1	1	2	2	9	11	3
65≤	10	10	0	0	0	0	0	0	0	10	0
Total	62	50	6	1	1	2	2	5	19	34	4

Table 1 showed that the most affected age group were (15- 44 and 45- 64) (52/62). Ductal carcinoma was the prevalent (50/ 62) followed by the lobular carcinoma (6/ 62). Most of the tumors were classified as grade II and III (53/ 62).

CONCLUSION

CK 18 is a promising tissue marker which may be used as a target for breast cancer therapy or in vaccination trials. Vimentin appear of no clinical value since it is expressed in all the cancerous tissues and most of the normal tissues.

Recommendations
A large scale study is highly recommended and it is better to study other parameters like the stage, the ethnic group and the geographical area of the study subjects.

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REFERENCES

[1] Frixione E. *Cell motility and the cytoskeleton* **2008**; 46 (2): 73–94.
[2] Doherty GJ, McMahon HT. *Annual Review of Biophysics* **2008**; 37: 65–95.
[3] Minton AP. *Biophys. J* **1992**: 63 (4): 1090–100.
[4] Oshima R G, Millan J L, Cecena G. *Differentiation* **1986**; 33: 61-68.
[5] Leube R E, Bosch F X, Romano V, Zimbelmann R, Hofler H, Franke W W. *Differentiation* **1986**; 33: 69-85.
[6] Ku N O, Omary M B. (1994). *J. Cell Biol* **1994**; 127: 161-171.
[7] Waseem A, Karsten U, Leigh I M, Purkis P, Waseem N H, Lane E B. *Biochemistry* **2004**; 43: 1283-1295.
[8] Wang L, Srinivasan S, Theiss A L., Merlin D, Sitaraman S V. *J. Biol. Chem*. **2007**; 282: 8219-8227.
[9] Toivola D M, Ku N O, Resurreccion E Z, Nelson D R, Wright T L, Omary M B. *Hepatology* **2004**; 40: 459-466.
[10] Chang L, Shav-Tal Y, Trcek T, Singer RH, Goldman RD. *J Cell Biol* **2006**; **172** (5): 747–58.
[11] Sommers C L, Walker-Jones D, Heckford S E, Worland P, Valverius E, Clark R, McCormick F, Stampfer M, Abularach S, Gelmann E P. *Cancer Res* **1989**; 49: 4258-4263.
[12] Perreau J, Lilienbaum A, Vasseur M, Paulin D. *Gene* **1988**; 62: 7-16.
[13] Goldman R D, Khuon S, Chou Y, Opal P, Steinert P. *J Cell Biol* **1996**; 134 (4): 971–83.
[14] Sarria A J, Panini S R, Evans R M. *J Biol Chem* **1992**; 267 (27): 19455–63.
[15] Leader M, Collins M, Patel J, Henry K. *Histopathology* **1987**; 11 (1): 63–72.
[16] Moinova, Helen. *Cancer Epidemiology Biomarkers Prev* **2012**; 21 (4): 594–600.
[17] Vora HH, Patel NA, Rajvik KN, Mehta SV, Brahmbhatt BV, Shah MJ, Shukla SN, Shah PM. *Int J Biol Markers* **2009**; 24(1): 38-46.
[18] Enrique Lerma, Gloria Peiro, Teresa Ramo´n, Sonia Fernandez, Daniel Martinez, Cristina Pons, Fina Mun˜ oz, Josep Ma Sabate, Carmen Alonso, Bele´n Ojeda, Jaime Prat, Agustı´ Barnadas. *Modern Pathology* **2007**; 20: 1200–1207.
[19] Ute Woelfle, Guido Sauter, Sonja Santjer, Ruud Brakenhoff, Klaus Pantel. *Clinical Cancer Research* **2004**; 10: 2670- 2674.
[20] Verma S, Bal A, Joshi K, Arora S, Singh G. *APMIS* **2012**; 120 (12): 1008- 19.
[21] Victor E. Gould, George K. Koukoulis,Desiree S. Jansson,t Raymond B. Nagle,Werner W. Franke, Roland Molill. *American Journal of Pathology* **1990**; 137 (5): 1143- 1155.
[22] David J Dabbs**,** Mamatha Chivukula, Gloria Carter, Rohit Bhargava. *Modern Pathology* **2006**; 19: 1506- 1511.
[23] M Heatley, C Whiteside, P Maxwell, P Toner. *J Clin Pathol* **1993**; 46: 441-445.
[24] Seshadri R, Raymond WA, Leong AS, Horsfall DJ, McCaul K. (1996). *Int J Cancer* **1996**; 29;67(3): 353-6.
[25] Essa TM, el Tatawi FA, Hamdi KN, Arafa WA. *J Egypt Soc Parasitol* **1996**; 26 (2): 433-42.
[26] Kusinska RU, Kordek R, Pluciennik E, Bednarek AK, Piekarski JH, Potemski P. (**2009**). *J Exp Clin Cancer Res* **2009**; 20 (28) :118. doi: 10.1186/1756-9966-28-118.
[27] Domagala W, Markiewski M, Kubiak R, Bartkowiak J, Osborn M. *Virchows Arch A Pathol Anat Histopathol* **1993**; 423(6): 497-502.
[28] Niveditha SR, Bajaj P. *Indian J Pathol Microbiol* **2003**; 46(4): 579-84.

[29] Miyake Y, Hirokawa M, Norimatsu Y, Kanahara T, Monobe Y, Ohno S, Miyamoto T, Yakushiji H, Sakaguchi T, Aratake Y, Ohno E. *Diagn Cytopathol* **2009**; 37(6): 393-6.