



International Journal of
Cancer Research

ISSN 1811-9727



Academic
Journals Inc.

www.academicjournals.com

Correlation between CD34 Marker and Clinico-Pathologic Characteristics of Gastric Cancer

¹Afshin Habibi, ¹Nastaran Ranjbari, ²Fakher Rahim and ¹Nematollah Jazayeri

¹Department of Pathology, Jundishapur University of Medical Sciences, Ahvaz, Iran

²Hearing Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Corresponding Author: Nastaran Ranjbari, Department of Pathology, Jundishapur University of Medical Sciences, Ahvaz, Iran Tel/Fax: +986133367562

ABSTRACT

One of the most common cancers in the world, especially in underdeveloped countries, is Gastric Cancer (GC) which kills thousands of people annually. It is also prevalent in our country, Iran and according to statistics, it is the most common cancer in men. Since, the relationship between expression of CD34 cell surface markers and many cancers has been approved in the present study, the marker expression association with GC is studied. In this study, a total of 47 paraffin blocks of patients with GC referring to Imam Khomeini hospital in Ahvaz, during 2003-2013 were used. The mean age of 47 patients, including, 35 men (74.5%) and 12 women, was 60±16.05 years old ranging from 24-84. The mean tumor size was 34.5 ranging from 2-12 cm. More than 71% of tumors were intestinal type and the depth of more than 74% of them was in T3 phase. Incidence of marker was positive in only 8 patients (17%) and in rest 39 was negative. The CD34 expression was significantly correlated only with the tumor type ($p = 0.00$), degree of malignancy ($p = 0.00$) and neural invasion ($p = 0.01$). In this study, a positive correlation was observed between the marker expression and three factors of tumor type, degree of differentiation and neural invasion. It was also concluded that due to the negative CD34 expression in 83% of samples, the absence of positive CD34 cells in tumor tissue can be considered as a marker in GC, although it is recommended to do further research with more samples in this context.

Key words: Adenocarcinoma, CD34, tumor, gastric cancer

INTRODUCTION

Gastric Cancer (GC) is one of the most common cancers in the world, which accounts for 7% of all cancers (Kamangar *et al.*, 2006). The GC also accounts for 9% of all cancer-related deaths in the world (Kamangar *et al.*, 2006). The GC mortality rate is increasing in the world and its estimated incidence be more than one million new cases per year in the world (De Martel *et al.*, 2013; Kelley and Duggan, 2003). Around fifty thousand new cases of GC are reported In Iran (Malekzadeh *et al.*, 2009). The gender-specific incidence of this cancer varies in different regions of the world, which is reported as 26.1 in men and 11.1 in women per one hundred thousand people in Iran. The highest incidence of GC can be seen in North America, East Europe and parts of the Middle East in which Iran is one of its countries (De Martel *et al.*, 2013; Kelley and Duggan, 2003; Malekzadeh *et al.*, 2009). It is reported that in the United States, the ratio of GC in men is two times more than women (Conteduca *et al.*, 2013). The ratio in Iran is about three times (Malekzadeh *et al.*, 2009). The disease typically starts in the fourth decade of life and its prevalence

increases with aging and the highest prevalence occurs in the seventh decade of life in men and particularly older ages in women (Conteduca *et al.*, 2013; Cunningham *et al.*, 2006; Kelley and Duggan, 2003). The mean age in Iran was 50-60 years old and approximately 75% of patients have referred in the advanced stages of metastatic (Malekzadeh *et al.*, 2009).

The GC is in fact the out of control growth of malignant cells in the stomach, which origin is usually the gastric mucosa cells and it is of those cancers that grow slowly over the years. But before the cancer is really occurred, some changes are caused in the stomach linings. If it is early diagnosed, it can be completely cured and if it is late diagnosed, it may spread to other parts of the stomach (Roshanaei *et al.*, 2011).

Gastric adenocarcinoma is the most common cancer which is divided into two types: intestinal and diffuse (based on the Lawrence classification). Intestinal adenocarcinoma is more common and it is thought to originate from the neoplastic epithelium. Diffuse adenocarcinoma are made through the growth of signet ring tumor cells and further involves the deeper layers of stomach and unlike intestinal adenocarcinoma, tumor formation is rare. According to studies, prognosis of intestinal type is better than diffuse (Rosai, 2011).

In recent decades, cell surface biomarkers play a major role in determining the prognosis and response to treatment of cancers, including GC. One of these markers is CD34, which is a cell surface glycoprotein with a weight of 110 kDa (Deguchi and Komada, 2000; Greaves *et al.*, 1992) and it originates from hematopoietic stem cells (Simmons *et al.*, 1992). Phosphorylated by kinases, CD34 is activated in the cell membrane and thus play a role in signaling (Sidney *et al.*, 2014).

CD34-positive stromal cells are scattered all over the body including the gastrointestinal tract (Narvaez *et al.*, 1996; Yamazaki and Eyden, 1995) and play a role in the differentiation and proliferation of epithelial and mesenchymal stem cells as well as mediating for immune responses (Kuroda *et al.*, 2004; Yamazaki and Eyden, 1995, 1996).

The CD34 expression is significantly associated with diffuse-type gastric adenocarcinoma, while CD34 was completely negative in colonic adenocarcinoma (Nakayama *et al.*, 2001). Also, patients with GC receiving chemotherapy, who have a higher percentage of positive CD34 as well as CD34-positive/vwf, have lower survival rates than others (Shin *et al.*, 2008). Numerous studies have used the expression of this protein as a reliable marker in cancerous tumors by immunohistochemistry. For example, in GC (Dvorak, 1986; Hayashi, 1975), colon (Moreira *et al.*, 2011; Qasim *et al.*, 2012), liver (Cui *et al.*, 1996), blood (Borowitz *et al.*, 1990) and lung (Van de Rijn *et al.*, 1994), the CD34 expressions have been used for diagnostic examination, as well as the relationship between these markers and clinico-pathologic factors.

Thus, the importance of cells containing CD34 is that their diffusion and placement in tissues can be considered as a criterion and marker for tumor diagnosis and study. This study intended to examine CD34 expression in GC to clarify the importance of CD34-positive stromal cells in tumor stromal structure and its role in the development of GC, according to its histologic type.

MATERIALS AND METHODS

Study design and population: This descriptive study was conducted on all gastric pathological samples of patients undergoing GC treatment in Imam Khomeini hospital, Ahwaz. Sample includes 47 patients diagnosed with early GC, who underwent surgical treatment of gastric adenocarcinoma in Imam Khomeini Hospital during the years 2003-2013.

Method: Gastric samples were not fixed in 10% buffered formalin and were embedded in paraffin. Three slices with a thickness of 4 mm were prepared from paraffin block and then, each sample

underwent haematoxylin-eosin (H and E) conventional staining and immunohistochemical (IHC) staining with CD34 marker and was finally evaluated. Histological type was sections according to Lauren classification (Lauren, 1965). Immunohistochemical study for CD 34 antigen was performed using the avidin-biotin-peroxidase complex technique on formalin-fixed and paraffin-embedded tumor sections (Tenderenda *et al.*, 2001). Then tissue sections after the enzyme digestion were treated with monoclonal anti-CD34 antibody (diluted 1:25, overnight, Novocastra Laboratories, UK).

Statistical analysis: Clinico-pathologic factors for each sample were determined, including tumor size, tumor type, malignancy degree, depth of tumor invasion, neural invasion, vascular invasion and lymph nodes invasion. In addition, demographic data (patient age and sex) were determined based on the patient record. Finally, all results were evaluated using SPSS 20 and through Fisher's exact tests and Chi-square test.

RESULTS

Out of 47 samples with GC, 35 were male (74.5%) and 12 female (25.5%). All patients were diagnosed with GC and a significant difference was observed between the two groups ($p = 0.01$). Patients average age was 60 ± 16.05 in the range of 24-84, out of which 6 (12.8%) were under 40 years, 7 (15%) between 40-50 years, 8 (17%) in the range of 50-60 years, 9 (19%) in the range of 60-70 and 17 (36.2%) above 70 years old. The mean age of men and women was 61.3 ± 15.67 and 56.2 ± 17.2 , respectively. The mean tumor size of patients was 34.5 cm with a range of 2-12 cm. The mean tumor size in patients with negative CD34 expression was 6.08 and in positive patient was 62.6 mm. No significant difference was observed between tumor size and CD34 expression ($p = 0.45$). In terms of tumor types, 33 (71.7%) had intestinal tumor and 13 (28.3%) had diffuse-type tumor, which difference was significant only at 5% level ($p = 0.03$). The status of one person (2.1%) was undetermined in terms of tumor type. The CD34 markers expression had a significant difference in terms of tumor location ($p = 0.00$).

Eleven (23.4%) out of total population were well differentiated in terms of the degree of tumor, 15 (31.9%) were moderately differentiated and 21 (44.7%) were poorly differentiated and their difference was not significant ($p = 0.19$). A significant difference was observed in terms of marker expression among the three groups ($p = 0.00$). The mean age of patients, who were negative in terms of CD34 marker was 62.4 ± 14.8 and those who were positive in term of CD34 marker was 49.6 ± 19.15 . The CD34 marker expression had no significant difference in terms of age ($p = 0.84$). The results indicated that the CD34 marker is directly linked with the type of GC (Table 1), so that more than 87% of the positive cases had the diffuse type.

In terms of depth of tumor invasion, 1 patient (2%) was in T1, 10 (21.3%) in T2, 35 (75.5%) in T3 and 1 (2%) in T4 and their difference was also significant ($p = 0.00$). The CD34 expression was not significant in terms of tumor depth ($p = 0.06$). Twenty four patients (51%) had lymphatic invasion and 23 (49%) lacked it, which the difference between them was not significant ($p = 0.88$). Marker expression was not significant in terms of invasion ($p = 0.89$). Investigation of vascular invasion showed that 17 patients (36.1%) had the invasion and 30 (63.9%) lacked the vascular invasion that in this regard, no significant difference was observed ($p = 0.06$). No significant difference exists between CD34 expression and vascular invasion ($p = 0.93$). Then patients were evaluated in terms of neural invasion, that 30 (63.9%) of them had the neural invasion and 17 (36.1%) lacked a lymphatic invasion and the difference was not significant ($p = 0.06$). Nevertheless, the CD34 marker expression showed a significant difference in this regard ($p = 0.01$).

Table 1: Frequency of patients and CD34 expression in terms of clinico-pathologic characteristics of patients with cancer

Variables and subgroups	No.	%	CD34 expression				p-values
			Positive		Negative		
			No.	%	No.	%	
Gender							
Man	35	74.5	5	62.5	30	77.00	0.39
Woman	12	25.5	3	37.5	9	23.00	
Patient age							
Under 50	13	27.6	3	37.5	10	25.60	0.84
50-70	17	36.2	4	50.0	13	33.30	
70 and higher	17	36.2	1	12.5	16	41.10	
Tumor size							
<4 cm	12	25.5	1	12.5	11	28.20	0.45
4-6 cm	16	34.0	2	25.0	14	35.90	
6-8 cm	10	21.3	2	25.0	8	20.50	
>8 cm	9	19.1	3	37.5	6	15.40	
Type of tumor							
Intestinal	33	71.7	1	12.5	32	82.00	0.00
Diffuse	13	28.3	7	87.5	7	18.00	
Degree of differentiation							
Good	11	23.4	0	0.0	11	28.20	0.00
Average	15	31.9	0	0.0	15	38.50	
Weak	21	44.7	8	100.0	13	33.30	
Depth of invasion							
T1	1	2.00	0	0.0	1	2.00	0.06
T2	10	21.3	0	0.0	0	25.70	
T3	35	74.5	7	87.5	28	71.80	
T4	1	2.00	1	12.5	0	0.00	
Lymphatic invasion							
Yes	24	51.0	4	50.0	20	51.30	0.89
No	23	49.0	4	50.0	19	48.70	
Vascular invasion							
Yes	17	36.1	3	37.5	14	35.90	0.93
No	30	63.9	5	62.5	25	64.10	
Neural invasion							
Yes	30	63.9	8	100.0	22	56.44	0.01
No	17	36.1	0	0.0	17	43.60	

DISCUSSION

This study that was performed to examine CD34 expression in GC to clarify the importance of CD34-positive stromal cells in tumor stromal structure and its role in the development of GC, according to its histologic type and found that CD34 expression is correlated with the tumor type, the degree of differentiation and neural invasion.

Most patients (75%) were over the age of fifty. However, there were cases of GC at an early age. With regard to sexual preference of 3-1 for men in the incidence of GC, the ratio obtained in this study is quite consistent with past results in Iran (Malekzadeh *et al.*, 2009). However, this ratio is 2-1 in America (Conteduca *et al.*, 2013), which may be due to a variety of environmental factors affecting the incidence of cancer in different regions of the world.

The results indicated that the CD34 marker is directly linked with the type of GC, so that more than 87% of the positive cases had the diffuse type. The results were fully consistent with the study by Nakayama *et al.* (2001). In the study, from 55 cases (15 diffuse and 40 intestinal types) of GC, all 40 cases of intestinal adenocarcinoma were negative CD34, while 8 out of 15 samples of diffuse adenocarcinoma were CD34-positive. These positive cases included those involving the muscular layer and sub-serous. In the early stages of GC, CD34 expression was seen in none of the intestinal

and diffuse type, while in advanced adenocarcinoma, this marker was only found in diffuse tumors (Rosai, 2011). Nakayama *et al.* (2002) have reported similar results in examining the distribution and role of CD34-positive cells in 74 patients with GC.

In this study, the CD34 marker expression also was associated with tumor malignancy grade. More than 61% of poorly differentiated tumors were CD34-positive, while all tumors with good and moderate malignancy had a negative expression. In a study which had results completely similar to the present study (Tenderenda *et al.*, 2001) conducted a lab trial and evaluated the expression of CD34 in 58 samples of GC and its correlation with histology, stage, proliferation, p53 expression and apoptosis index and a significant relationship was obtained between CD34 expression and two main histological parameters, tumor malignancy degree and histological type. In moderately differentiated tumors, CD34 expression was significantly lower than less differentiated cancers and CD34 expression in diffuse type was more than intestinal one. Similar results were reported on the positive relationship between this marker and differentiation and tumor type on the intestinal adenocarcinoma in numerous studies (Choi *et al.*, 2009; Feng *et al.*, 2010; Nakayama *et al.*, 2000). Although, the depth of invasion was not significantly different from marker expression but the difference was too much, so that 87.5% of positive cases had a depth of T3 that of all tumors in depth of T3, a quarter of them have expressed a marker. Also our observations showed no relationship between this marker and gender, age, tumor size, lymph node invasion and vascular invasion. Likewise, a lack of correlation between CD34 marker and clinical and pathological factors including; lymphatic invasion, gender, age and stage of differentiation were also confirmed in the study by Wang *et al.* (2004). In the study of Ahmed *et al.* (2010), as well as, Nosrati *et al.* (2014) on colorectal cancer, no association was reported between the CD34 expression and clinicopathologic factors. In contrast, increasing the CD34 marker expression was positively correlated with tumor stage, infiltration and lymph node and therefore, it was concluded that this marker in GC is correlated within the disease development (Chen *et al.*, 2008). In the case of neural invasion, the association of CD34 with neural invasion was clear, so that all CD34-positive patients were also positive in terms of neural invasion.

Given the observations, 83% of cases of cancer were negative in term of CD34 expression. This result leads us to this point that CD34-positive cells might be negatively correlated with gastric adenocarcinoma. In a study of Li *et al.* (2014), the percent of CD34-positive cells in normal tissues around tumor was reported to be more than the tumor area, which is due to hemodialysis changes and the vital role of this marker in maintenance of tumor mass angiogenesis. Also, Kuroda *et al.* (2004) studied the CD34-positive cells diffusion in intestinal tumors and concluded that stromal cells of CD34-positive, present in the area between sub-mucosal and sub-serous of normal tissue, were removed from this region after tumor invasion and were transferred to deeper layers of tumors. The results were confirmed in another study that showed the inflamed tissue in tumor margins and tumor stroma of stromal cells are free of CD34 (Nakayama *et al.*, 2000).

CONCLUSION

In this study, we found that the expression of CD34- positive is correlated with the tumor type, the degree of differentiation and neural invasion, as well as with tumor depth, although non-significantly. Therefore, by evaluating this marker in more GC samples, we can achieve more comprehensive information. In addition, the negative correlation of these markers with more factors can be a reason for the inverse relationship of CD34-positive cells with gastric adenocarcinoma tumors.

REFERENCES

- Ahmed, M.A., D. Jackson, R. Seth, A. Robins, D.N. Lobo, I.P. Tomlinson and M. Ilyas, 2010. CD24 is upregulated in inflammatory bowel disease and stimulates cell motility and colony formation. *Inflamm. Bowel Dis.*, 16: 795-803.
- Borowitz, M.J., J.J. Shuster, C.I. Civin, A.J. Carroll and A.T. Look *et al.*, 1990. Prognostic significance of CD34 expression in childhood B-precursor acute lymphocytic leukemia: A Pediatric Oncology Group study. *J. Clin. Oncol.*, 8: 1389-1398.
- Chen, L., X. Li, G.I. Wang, Y. Wang, Y.Y. Zhu and J. Zhu, 2008. Clinicopathological significance of overexpression of TSPAN1, K167 and CD34 in gastric carcinoma. *Tumori*, 94: 531-536.
- Choi, D., H.W. Lee, K.Y. Hur, J.J. Kim and G.S. Park *et al.*, 2009. Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. *World J. Gastroenterol.*, 15: 2258-2264.
- Conteduca, V., D. Sansonno, G. Lauletta, S. Russi, G. Ingravallo and F. Dammacco, 2013. *H. pylori* infection and gastric cancer: State of the art (Review). *Int. J. Oncol.*, 42: 5-18.
- Cui, S., H. Hano, A. Sakata, T. Harada, T. Liu, S. Takai and S. Ushigomey, 1996. Enhanced CD34 expression of sinusoid like vascular endothelial cells in hepatocellular carcinoma. *Pathol. Int.*, 46: 751-756.
- Cunningham, D., W.H. Allum, S.P. Stenning, J.N. Thompson and C.J. Van de Velde *et al.*, 2006. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N. Engl. J. Med.*, 355: 11-20.
- De Martel, C., D. Forman and M. Plummer, 2013. Gastric cancer: Epidemiology and risk factors. *Gastroenterol. Clin. North Am.*, 42: 219-240.
- Deguchi, T. and Y. Komada, 2000. Homing-associated cell adhesion molecule (H-CAM/CD44) on human CD34⁺ hematopoietic progenitor cells. *Leukemia Lymphoma*, 40: 25-37.
- Dvorak, H.F., 1986. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N. Engl. J. Med.*, 315: 1650-1659.
- Feng, S.T., C.H. Sun, Z.P. Li, H.K.F. Mak, Z.P. Peng, H.Y. Guo and Q.F. Meng, 2010. Evaluation of angiogenesis in colorectal carcinoma with multidetector-row CT multislice perfusion imaging. *Eur. J. Radiol.*, 75: 191-196.
- Greaves, M.F., J. Brown, H.V. Molgaard, N.K. Spurr, D. Robertson, D. Delia and D.R. Sutherland, 1992. Molecular features of CD34: A hemopoietic progenitor cell-associated molecule. *Leukemia*, 6: 31-36.
- Hayashi, H., 1975. [Computer-controlled frequency synthesizer for electrophysiological experiments]. *J. Physiol. Soc. Jpn.*, 37: 137-138, (In Japanese).
- Kamangar, F., G.M. Dores and W.F. Anderson, 2006. Patterns of cancer incidence, mortality and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. *J. Clin. Oncol.*, 24: 2137-2150.
- Kelley, J.R. and J.M. Duggan, 2003. Gastric cancer epidemiology and risk factors. *J. Clin. Epidemiol.*, 56: 1-9.
- Kuroda, N., H. Nakayama, E. Miyazaki, Y. Hayashi, M. Toi, M. Hiroi and H. Enzan, 2004. Distribution and role of CD34-positive stromal cells and myofibroblasts in human normal testicular stroma. *Histol. Histopathol.*, 19: 743-751.
- Lauren, P., 1965. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. *Acta Pathol. Microbiol. Scand.*, 64: 31-49.
- Li, K., Z. Dan and Y.Q. Nie, 2014. Gastric cancer stem cells in gastric carcinogenesis, progression, prevention and treatment. *World J. Gastroenterol.*, 20: 5420-5426.

- Malekzadeh, R., M.H. Derakhshan and Z. Malekzadeh, 2009. Gastric cancer in Iran: Epidemiology and risk factors. *Arch. Iran. Med.*, 12: 576-583.
- Moreira, L.R., A.A. Schenka, P. Latuf-Filho, A.L. Penna and S.P. Lima *et al.*, 2011. Immunohistochemical analysis of vascular density and area in colorectal carcinoma using different markers and comparison with clinicopathologic prognostic factors. *Tumour Biol.*, 32: 527-534.
- Nakayama, H., H. Enzan, E. Miyazaki, N. Kuroda, K. Naruse and M. Hiroi, 2000. Differential expression of CD34 in normal colorectal tissue, peritumoral inflammatory tissue and tumour stroma. *J. Clin. Pathol.*, 53: 626-629.
- Nakayama, H., H. Enzan, E. Miyazaki, N. Kuroda and K. Naruse *et al.*, 2001. CD34 positive stromal cells in gastric adenocarcinomas. *J. Clin. Pathol.*, 54: 846-848.
- Nakayama, H., H. Enzan, E. Miyazaki and M. Toi, 2002. α Smooth muscle actin positive stromal cells in gastric carcinoma. *J. Clin. Pathol.*, 55: 741-744.
- Narvaez, D., J. Kanitakis, M. Faure and A. Claudy, 1996. Immunohistochemical study of CD34-positive dendritic cells of human dermis. *Am. J. Dermatopathol.*, 18: 283-288.
- Nosrati, A., F. Naghshvar, Z. Torabizadeh and F. Salehi, 2014. Correlation of Colorectal cancer stem cell marker CD24 expression with clinicopathologic features and survival of patients with colorectal cancer. *Govaresh*, 19: 86-94.
- Qasim, B.J., H.H. Ali and A.G. Hussein, 2012. Immunohistochemical expression of PCNA and CD34 in colorectal adenomas and carcinomas using specified automated cellular image analysis system: A clinicopathologic study. *Saudi J. Gastroenterol.*, 18: 268-276.
- Rosai, J., 2011. *Rosai and Ackerman's Surgical Pathology*. 10th Edn., Mosby, St. Louis, MO., USA., ISBN-13: 9780323069694, Pages: 2892.
- Roshanaei, G., A. Kazemnejad and S. Sadighi, 2011. Survival estimating following recurrence in gastric cancer patients and its relative factors. *Koomesh*, 12: 223-228.
- Shin, S.J., H.C. Jeung, J.B. Ahn, S.Y. Rha and N.C. Yoo *et al.*, 2008. Mobilized CD34⁺ cells as a biomarker candidate for the efficacy of combined maximal tolerance dose and continuous infusional chemotherapy and G-CSF surge in gastric cancer. *Cancer Lett.*, 270: 269-276.
- Sidney, L.E., M.J. Branch, S.E. Dunphy, H.S. Dua and A. Hopkinson, 2014. Concise review: evidence for CD34 as a common marker for diverse progenitors. *Stem Cells*, 32: 1380-1389.
- Simmons, D.L., A.B. Satterthwaite, D.G. Tenen and B. Seed, 1992. Molecular cloning of a cDNA encoding CD34, a sialomucin of human hematopoietic stem cells. *J. Immunol.*, 148: 267-271.
- Tenderenda, M., P. Rutkowski, D. Jesionek-Kupnicka and R. Kubiak, 2001. Expression of CD34 in gastric cancer and its correlation with histology, stage, proliferation activity, p53 expression and apoptotic index. *Pathol. Oncol. Res.*, 7: 129-134.
- Van de Rijn, M., C.M. Lombard and R.V. Rouse, 1994. Expression of CD34 by solitary fibrous tumors of the pleura, mediastinum and lung. *Am. J. Surg. Pathol.*, 18: 814-820.
- Wang, M.C., Y.M. Yang, X.H. Li, F. Dong and Y. Li, 2004. Maspin expression and its clinicopathological significance in tumorigenesis and progression of gastric cancer. *World J. Gastroenterol.*, 10: 634-637.
- Yamazaki, K. and B.P. Eyden, 1995. Ultrastructural and immunohistochemical observations on intralobular fibroblasts of human breast, with observations on the CD34 antigen. *J. Submicrosc. Cytol. Pathol.*, 27: 309-323.
- Yamazaki, K. and B.P. Eyden, 1996. Ultrastructural and immunohistochemical studies of intralobular fibroblasts in human submandibular gland: The recognition of a CD34 positive reticular network connected by gap junctions. *J. Submicrosc. Cytol. Pathol.*, 28: 471-483.