e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(5); 738-745

Original Research Article

Expression of E-Cadherin in Colorectal Carcinoma: An Immunohistochemical & Histological Correlation

Swagatika Agrawal[1], Sunil Agarwal[2], Debi Prasad Mishra[3], Kamlesh lenka[4], G Punyatoya[5]

[1]Associate Professor, Dept. Pathology, Hi Tech Medical College, Bhubaneswar
[2]Associate Professor, Dept. Pathology, Hi Tech Medical College, Rourkela
[3] Professor, HOD Dept. Pathology, Hi Tech Medical College, Bhubaneswar
[4]PG (3-Year) Dept. Pathology, Hi Tech Medical College, Bhubaneswar
[5]PG (3-Year) Dept. Pathology, Hi Tech Medical College, Bhubaneswar

Received: 25-03-2023 / Revised: 25-04-2023 / Accepted: 15-05-2023
Corresponding author: Dr. Swagatika Agrawal
Conflict of interest: Nil

Abstract

Background: Usually cancer in colon or rectum which arises from benign polyps, show variable clinicopathological features.

Aim: To evaluate the E cadherin expression in colorectal carcinoma.

Material and Method: A hospital based prospective study was conducted in eastern India. All the cases of colorectal carcinoma undergone colonoscopy and surgery were included. Result and data were analyzing in the department of pathology of Hi-Tech Medical College Bhubaneswar between 2018-2020 for diagnostic accuracy by using data.

Results: Out of 59 cases mostly elderly age group and males were more affected. E cadherin expression decrease with increasing grade.

Conclusion: E cadherin expression can be used for investigate the tumor invasion, metastasis, better treatment and more comprehensive management of patients.

Keyword: Colorectal Carcinoma, E-Cadherin, Evaluate, Metastasis, Diagnostic Accuracy.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Colorectal cancer is an imposing problem worldwide. It is the third most common cancer in men (663000 cases, 10.0% of all cancer cases) and the second most common in women (571000 cases, 9.4% of all cancer cases) [1]. Almost 60% of cases are seen in developed countries. The number of Colorectal cancer-related deaths is approximated to be close to 6,08,000 worldwide, accounting for 8% of all cancer deaths and making Colorectal cancer the fourth most common cause of death by cancer. In India, the annual incidence rates for colon cancer and rectal cancer in men are 4.4 and 4.1 per 100000, respectively. The Annual incidence rates for colon cancer in women is 3.9 per 100000. Colon cancer ranks 8th and rectal cancer ranks 9th among men. For women, rectal cancer does not rank within the top 10 cancers, meanwhile colon cancer ranks 9th [2].A review of the 12 population-based cancer registries from India (Benaguluru, Barshi, Bhopal, Chennai, Delhi, Mumbai, Ahmedabad, Karunagappally, Kolkata, Nagpur, Pune, and Thiruvananthapuram) again demonstrated that the incidence of colon and rectal cancer is significantly lower in India for both genders when compared to that of the West [3].

Unfortunately, India's low incidence rate is also associated with a low 5-year survival rate. Several factors have been identified as factors affecting the prognosis of patients. Several factors include age, sex, diet and conditions like FAP have proved to affect the incidence of colorectal carcinoma. One of these factors affecting prognosis of patients is E cadherin. E-cadherin is a very important cellular adhesion protein that is vital part of epithelial mesenchymal transition. EMT is a process by which epithelial cells lose their polarity of cells in the apico-basal region and lead to formation of migratory mesenchymal cells. There is down regulation of cellular adhesion proteins such as epithelial (E-) cadherin and γ-catenin. There is usefulness of EMT in organogenesis and regeneration. But EMT is also a part of the process that leads to tumour invasion and metastasis. E-cadherin is a transmembrane glycoprotein which connects epithelial cells together at adherens junctions. In normal cells, E-cadherin exerts its tumour suppressing role mainly by sequestering β-catenin from its binding to LEF (Lymphoid enhancer factor)/TCF (T cell factor) which serves the function of transcribing genes of the proliferative Want signaling pathway. Despite the ongoing debate on whether the loss of E-cadherin is the cause or effect of epithelial-mesenchymal transition (EMT), E-cadherin functional loss has frequently been associated with poor prognosis and survival in patients of various cancers. The dysregulation of E-cadherin expression that leads to carcinogenesis happens mostly at the epigenetic level but there are cases of genetic alterations as well. E-cadherin expression has been linked to the cellular functions of invasiveness reduction, growth inhibition, apoptosis, cell cycle arrest and differentiation [4]. Several studies including studies like Khoursheed MA et al and Palaghia M et al have shown the higher expression of E-cadherin in well differentiated and moderately differentiated cases than in cases of poorly differentiated colorectal cancer cases. These studies have also shown E cadherin expression affecting prognosis of patients. In this study the aim was to evaluate the expression of e cadherin in colorectal carcinoma and its relationship with clinicopathological parameters for prognostication in colorectal cancer.

Aims and Objective

The aim of the study:- is to assess the expression of E-cadherin in colorectal carcinoma is to correlate it's expression with histological grading and staging of the tumor.

Materials and Methods

The prospective study of Expression of E Cadherin in Colorectal Carcinoma-A Immunohistochemical and Histological Correlation- was carried out over a period of2 years from 2018 to 2020 in the P.G. Department of Pathology in collaboration with the Department of Surgery of Hi-Tech Medical college and Hospital, Bhubaneswar.

The study was approved by the Institutional Ethics Committee

Inclusion Criteria:

Patients included in our study were:
All the diagnosed cases of colorectal adenocarcinoma undergone colonoscopy and colorectal surgery

Exclusion Criteria:

1. l. All the cases of inflammatory bowel's disease
2. All the benign lesions, polyps and dysplastic conditions of bowel.
3. The relevant data at the time of presentation were noted in the department of pathology, in collaboration with department of surgery of Hi-Tech Medical College and Hospital, Bhubaneswar.

-Age, sex
-Mode of presentation e.g. rectal bleeding/alteration of bowel habit
All data were recorded in a carefully structured proforma

Immunohistochemistry: E Cadherin Status:

From the paraffin embedded blocks two section of 3µm were taken on poly-Lysine coated glass slides for immunohistochemical staining of E-cadherin. This process was carried out by using avidin biotin peroxidise technique. Antigen retrieval was performed using TRIS buffer solution at 640 ° for 30 minutes. Slides were incubated in 3% hydrogen peroxide. The primary Ab (mouse monoclonal Ab) for E-cadherin had specificity against the Ab in test was added to the section & the excess was washed off. The labelled secondary Ab which had specificity against an Ag determinant present on the primary Ab was then added; the bound antigen was visualized with 3,3-diaminobenzidine tetrahydrochloride solution as the chromogen and counter staining was done with H&E. In our case we had taken carcinoma breast for Her2/neu as positive control. Negative control was used to verify the specificity of the labelling of the target Ag by primary Ab. Negative external control was used by omitting the primary antibody and treated with phosphate buffered saline(PBS)

Evaluation (scoring) of E-cadherin staining-both cytoplasmic & membranous positive

Based on Allred scoring system

1. Negative – 0 positive cell
2. Weak - ≤ 1 %positive cell
3. Intermediate -1-10 %
4. Strong - 11-33 %

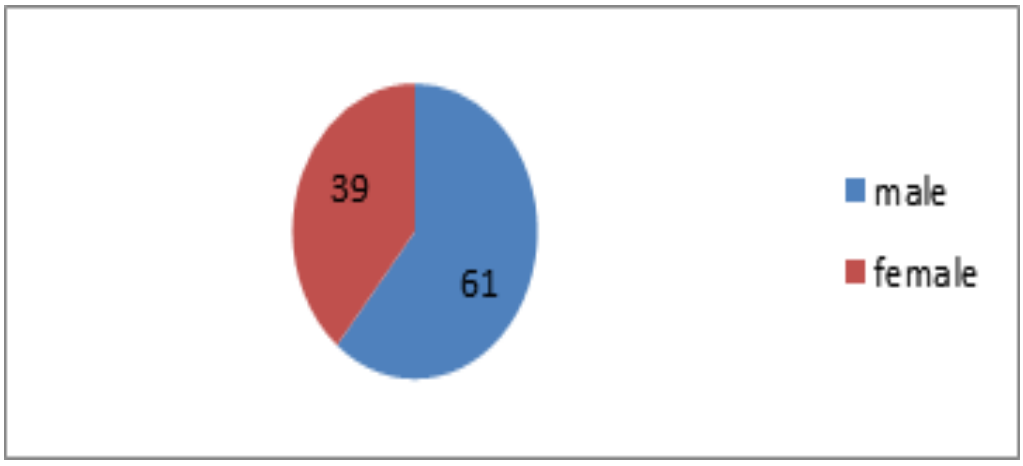
E- cadherin expression staining was scored as strong when immunoreactivity in the tumor region showed a similar membranous staining to its normal counterpart in more than 75% of the cells. Discontinuous membranous staining in 25-75% of the cells was scored as moderate staining. Absence of membranous staining or positive immunoreactivity in less than 25% of the cells was graded as weak or none.

Statistical Analysis

Statistical analysis was done to find out significance of E- cadherin expression in carcinoma and association between expression of e cadherin and various histopathological parameters. The relationship between qualitative parameters were determined using chi-square test, statistical significance was defined.

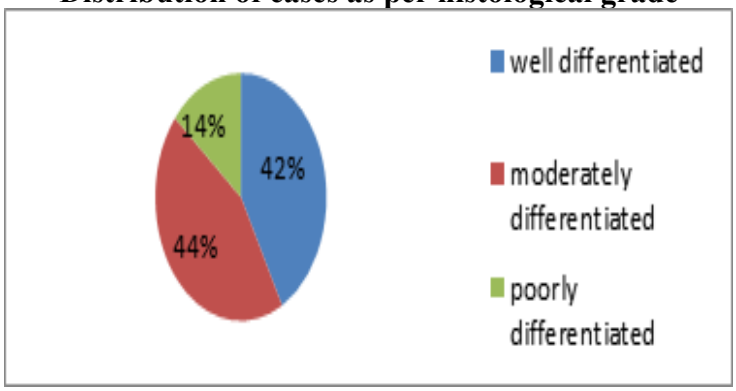
Results

Total no of cases included in study this study were 59. Distribution of cases according to age group maximum no of cases were seen in the age group 51-60. Colorectal carcinoma revealed male ratio was more than female

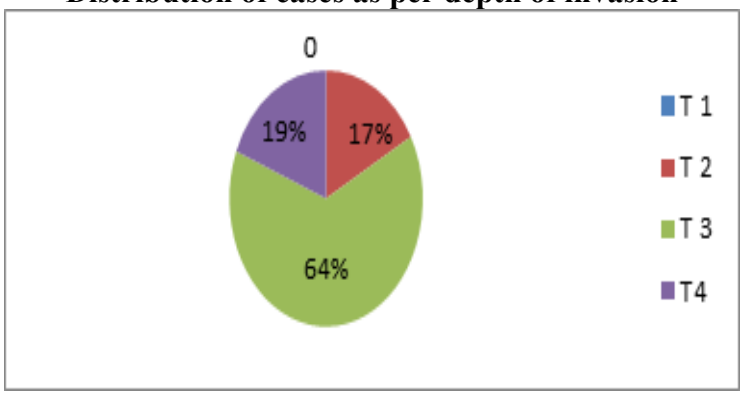


Distribution of cases as per site of tumor origin

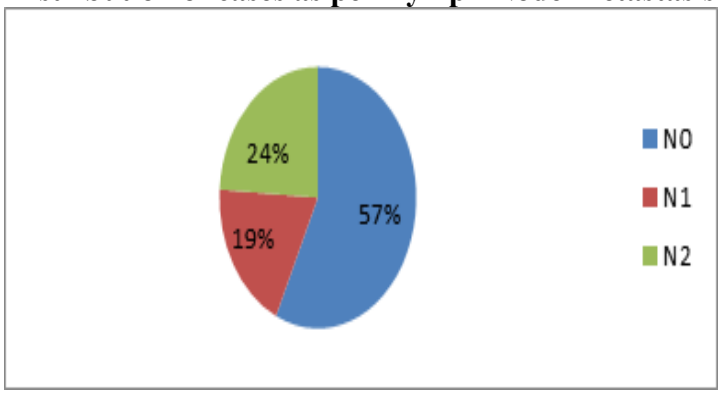
Distribution of cases as per histological grade



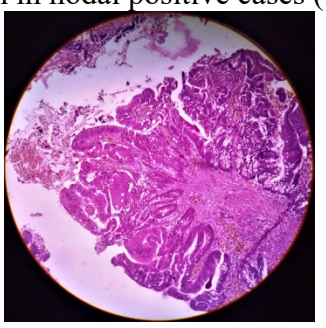
Distribution of cases as per depth of invasion

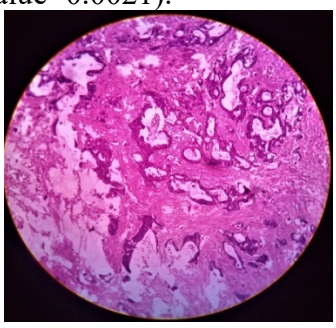


Distribution of cases as per Lymph Node Metastasis

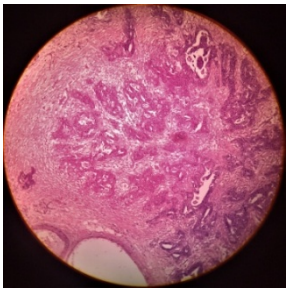
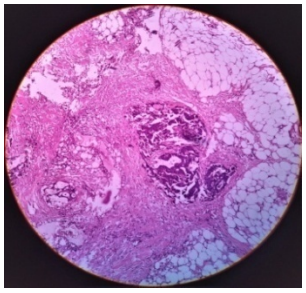


E cadherin expression more in cases of well and moderately differentiated carcinoma . these association of E-cadherin was statistically significant (p value-0.013).significant loss of E cadherin expression in nodal positive cases (p value- 0.0021).

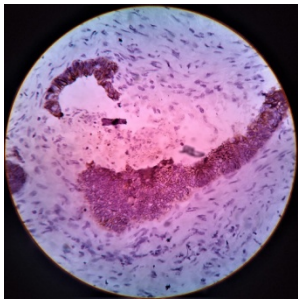
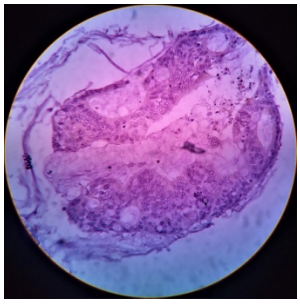
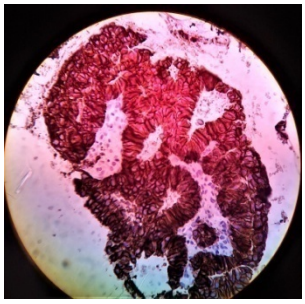




H-E Stained Section Showing Well Differentiated Colorectal Carcinoma **H-E Stained Section of Moderately Differentiated Carcinoma**

He Stained Sections Showing Tumor Deposits

Moderate Expression of E – Cadherin Negetive for E Cadherin Expression Strong E- Cadherin Expression

Discussion

Colorectal cancer is third most common cancer in men and second most common cancer in women. It is a major health problem in developed countries and also accounts for 8% of all cancer deaths.

Several factors have been indicated as the contributing factors for colorectal cancers. Low fiber and high fat diet have been seen to have a higher association with colorectal cancer. Several genetic factors have also been associated with colorectal cancers. Patients with FAP have a higher risk of developing colorectal cancer, which increases significantly after 50 years of age. Diseases like ulcerative colitis have also been shown to have a predisposing factor for development of colorectal cancer.

Early diagnosis and understanding of colorectal cancer in patients have led to better clinical outcome in association with surgical resection and chemotherapy. Several molecular markers aid in understanding of tumorigenesis, metastasis, and vascular invasion. Several molecular factors are also implicated with prognosis of the patient.

E cadherin is a form of calcium dependant adhesion molecule which plays a major role in epithelial adhesion .E cadherin play a vital role in migration of cells through a process known as epithelial mesenchymal transition. E-cadherin plays an important role in cellular adhesion. Decreased expression of E cadherin has often been associated increased invasiveness and local metastasis.

In current study we planned to evaluate the expression of E cadherin in colorectal cancer and its relation to the clinicopathological variables and grade and stage of disease.

Present study revealed the incidence of colorectal carcinoma was highest in the 5th to 7th decade ,which was similar to the results shown by studies by B Schuell et al 2006 [1], Manmeet Kaur et al 2011[2] Shoroq Mohamed Abas Al-Temimi 2014 [3] Kafah Hamdan Abdul Ghafour 2014 [4], Park et al 2014 [5] and Zhila Torabizadeh et al 2016 [6] ,found 50-60 years age group as most common age group for colorectal carcinoma. But B. Dalal A Elwy, et al 2012 [7] and Dr Sumitra A .Jain et al 2016 [8] found 40-50 age group as

most common age group for incidence colorectal carcinoma in their study.

In the present study there is no significant association of expression of e cadherin and the ages of the patient. This finding was found to be similar to the findings reported by kyungjinseo et al and JunjiGofuku et al.

In the present study there was also evaluation of incidence of colorectal carcinoma according to gender differentiation. The present study revealed 61 % of cases of colorectal carcinoma to be male and the rest of the 39% to be male. This male predominant incidence was similar to Renjie Wang et al, Petra Jurčić et al and kyungjinseo et al. In the present study there was no significant correlation between the gender of the patient and expression of E-cadherin and the results were similar in nature to findings of study by kyungjinseo et al.

In the present study moderately, differentiated carcinoma comprised of 49% of the net cases with well differentiated carcinoma coming in close second at 48% and poorly differentiated cases were 3% of the total cases. This higher presentation of colorectal carcinoma in the grade II that is moderately differentiated carcinoma was supported by previous studies conducted by Zohreh Niknami et al where moderately differentiated carcinoma comprised of 59% of the total cases and Khaled El Gehani et al where grade II carcinoma was 60% of the total cases. A significant association was also found between the expression of E-cadherin and the grade of the tumor. Well differentiated and moderately differentiated carcinoma expressed significantly E cadherin in higher percentage as compared to poorly differentiated cases. In the present study 22 out of 25 grade I cases were positive for colorectal carcinoma and 16 of the total 26 cases were positive for grade II carcinoma. In contrast only 3 of the 8 cases stained positive for grade III cases. These findings were supported by Dorudi et al. reported that ECAD expression is closely related to the stage and tumor grade, with more aggressive cancers displaying markedly reduced expression of ECAD Ghadimi et al [9] reported a significant relationship between reduced ECAD and lower tumor grade but did not find a clear correlation between loss of ECAD expression and depth of tumor infiltration into the intestinal wall (T-category) [10]. Recently, in a study of 140 stage II and III CRC cases, Ngan et al reported an inverse relationship between ECAD expression and tumor differentiation and showed that loss of expression of ECAD was an independent adverse prognostic factor in CRC [11].

We also evaluated the expression of e cadherin in colorectal carcinoma based on the staging of the disease. However no significant association was found between the staging of the disease and the expression of colorectal carcinoma. This was supported by previous studies as shown by kyungjinseo et al, Ranhong et al and further supported by Khaled El Gehani et al.

Upon analysis a significant association was found between lymph node status and lack expression of E cadherin in their primary site. We found higher lymph node status cases exhibited lower expression of e cadherin. This finding was supported by studies by Petra Juric et al and Kwak et al. Node-positive cancers exhibited significant loss of E-cadherin ($P<0.001$) according to Karamitopoulou *et al* [12]. Ozgüven *et al* [13] found that reduced E-cadherin expression was significantly associated with LN metastasis ($P=0.01$). A borderline association of E-cadherin expression and LN metastasis ($P=0.09$) was reported by Elzagheid *et al* [14]. Kim *et al* [15] reported that E-cadherin expression may serve as a predictive marker for tumor invasion and LN metastasis. However studies like Ran Hong et and Khaled El Gehani et al did not find any significant relationship between expression of E-cadherin in primary colorectal carcinoma sites and lymph node status of the patients. [16]

Conclusion

Relevant data exist regarding inverse relationship between grade of the tumor and expression of E-cadherin. Studies indicate that with increasing grade, the expression of E-cadherin decreases and subsequently affects the prognosis of the disease with poorly differentiated carcinoma exhibiting significant lack of E- cadherin expression as compared to the well differentiated and moderately differentiated counterparts.

Evidence exists to support the fact that expression of E cadherin is an independently predicts lymph node status in the patient and it can be used during the treatment period to better treat the patient and for more comprehensive management of patient in pre-operative and post-operative stages.

Future Prospective

A central role in EMT is by E-cadherin which is an epithelial cadherin that is a protein that deals with cellular adhesion. Loss of E cadherin molecule by cells is a hallmark indicator for EMT. Loss of E-cadherin in the process of EMT grants the cancer cells an ability to invade and metastasize to distant sites and further complicate the condition of the patient.

The prospect of targeted therapy to up regulate the epithelial adhesion molecules like E cadherin might prove helpful.

However expression of E cadherin in colorectal carcinoma did not show any significant relationship with age, gender or staging of the disease.

References

1. B Schuelll, T Gruenberger, W Scheithauerl, ChZielinskil and F Wrba3: HER 2/neu protein expression in colorectal cancer, BMC Cancer. 2006; 6: 123
2. Manmeet Kaur Gill, KalpanaJ ain, et al.: Expression of Her-2/neu in Colon Carcinoma and Its Correlation with the Histological Grades and the Lymph Nodes Status, 2011.
3. Shoroci Mohamad Abas Al-Temimi: Her2/ neu Expression in Colorectal Carcinoma and its correlation with Pathological Parameters by Immunohistochemistry, 2014 M.J.B. Journal, volume 8:2.
4. Kifah Hamdan Abdul Ghafour. Immunohisto chemical Expression of Her2/Neu Receptor in Human Colorectal Carcinoma (A Clinico pathological Study), The Iraqui post graduate joumal, 201.
5. Park DI, Kang MS, Oh SJ, Kim HJ, Cho YK, Sohn Cl, Buza N, Roque DM, Santin. HER2/neu in Endometrial Cancer. A Promising Therapeutic Target with Diagnostic Challenges. Archives of Pathology & Laboratory Medicine. March 2014; 138 (3): 343-50.
6. Zhila Torabizadehl, Anahita Nosrati, Shadi Tahvildari: Human Epidermal Growth Factor Receptor Expression in Colorectal Cancer and Its Relationship with Clinic opathological Characteristics.
7. Dalal A. Elwy, M.D.; Ahmed M. Abd El-Aziz, M.D.; Samar A. Elsheikh, M.D. And I-Ieba A. Ebrahim, M.B.B. Ch, Immunohisto chemical Expression
8. Dr. Sumita A. jainl, Dr.Rajnish m seena2, et a18Study of Her2/Neu Expression in Colon Adenocarcinoma and Its Correlation with Clinic opathological Variables, IOSR Journal of Dental and Medical Sciences (IOSR-JDMS): Feb. 2016; S61: 15, 2 Ver. VI.
9. Dorudi S, Sheffield JP, Poulsom R, Northover JM and Hart IR. E-cadherin expression in colorectal cancer. An immunocyto chemical and in situ hybridization study. Am J Pathol 1993; 142: 981-986.
10. Ghadimi BM, Behrens J, Hoffmann I, Haensch W, Birchmeier W, Schlag PM. Immunohistological analysis of E-cadherin, alpha-, beta- and gamma-catenin expression in colorectal cancer:

implications for cell adhesion and signaling. Eur J Cancer.
11. Ngan CY., et al. A multivariate analysis of adhesion molecules expression in assessment of colorectal cancer. Journal of Surgical Oncology 2007: 95.8:652-662.
12. Karamitopoulou E, Zlobec I, Kölzer V, Kondi-Pafiti A, Patsouris ES, Gennatas K, Lugli A. Proposal for a 10-high-power-fields scoring method for the assessment of tumor budding in colorectal cancer. Mod Pathol. 2013; 26: 295–301.
13. Ozgüven BY, Karaçetin D, Kabukçuoğlu F, Taşkin T and Yener Ş: Immunohistochemical study of E-cadherin and β-catenin expression in colorectal carcinomas. Pol J Pathol. 2011; 62:19–24.
14. Bendardaf R, Elzagheid A, Lamlum H, Ristamäki R, Collan Y, Pyrhönen S: E -Cadherin, CD44s and CD44v6 correlate with tumour differentiation in colorectal cancer.
15. Keum M, Lim S-B, Kim SA, Yoon YS, Kim CW, Yu CS, Kim JC. Clinicopathologic factors affecting recurrence after curative surgery for stage I colorectal cancer. J Korean Soc Coloproctol. 2012; 28 (1): 49–55.
16. Al-Rawee R., Al-Fathy D. M, & Bashir Alsabee, D. W. Delivering Integrated Health Care: Role and Importance of Multidisciplinary Team Clinic Role and Importance of Multidisciplinary Team Clinic in Nineveh Province . Journal of Medical Research and Health Sciences, 2022; 5(10): 2278–2294.