SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2023.v04i05.001

OPEN ACCESS

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Immunohistochemical Expression of CDX2 as Early Biomarkers for Gastric Intestinal Metaplasia among Sudanese Patients with Chronic Gastritis

Zeinab Ali Mohamed[1], Ibrahim B Elemam[1], Mohammed Abdelgader Elsheikh[1], Tibyan Abd Almajed Altaher[2], Ghanem Mohammed Mahjaf[3], Mosab Nouraldein Mohammed Hamad[4*]

[1]Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Shendi University, Sudan
[2]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Shendi University, Sudan
[3]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Sudan
[4]Assistant professor, Microbiology department, Faculty of Medicine, Elsheikh Abdallah Elbadri University, Sudan

***Corresponding Author:** Mosab Nouraldein Mohammed Hamad
Assistant professor, Microbiology department, Faculty of Medicine, Elsheikh Abdallah Elbadri University, Sudan

Article History: | Received: 09.08.2023 | Accepted: 12.09.2023 | Published: 13.09.2023 |

Abstract: ***Background:*** Chronic gastric infection with *Helicobacter pylori* can cause intestinal metaplasia, a pathologic change that is frequently a precursor of gastric cancer; CDX2 has an essential role in the development and maintenance of intestinal differentiation in the gut and ectopic sites such as intestinal metaplasia of the stomach. ***Objective*:** This study aimed to investigate caudal-type home box (CDX2) Immuno expression as an early indicator for gastric intestinal metaplasia among Sudanese patients with chronic gastritis. ***Materials and methods:*** This is a descriptive retrospective cross-sectional study conducted at Atbara Medical Complex and El-MakNimr University Hospital at River Nile State in the period from 2019 to 2022. 140 archival paraffin tissue blocks were collected from patients diagnosed with chronic gastritis, immunohistochemistry was used to detect (CDX2 expression as an early indicator for gastric intestinal metaplasia. ***Results:*** From 140 samples, 80 were females (57.1 %) and 60 were males (42.9%), the patient's ages ranged from (12 to 97) with a mean age of (53.27) years, and age was categorized into three groups, less than 40 (27) (19.3%), 40- 60 (64) (45.7%), more than 60 (49) (35.0%). The prevalence of gastric intestinal metaplasia was (17.9%, while no statistically significant correlation of gastric intestinal metaplasia with gender and age as the *P.value* was (0.308 and 0.256) respectively. ***Conclusion:*** The development of gastric intestinal metaplasia as a premalignant condition of gastric carcinoma is important to detect this change at an early point to prevent and control gastric cancer.

Keywords: CDX2, Gastric cancer, Immunohistochemical staining, H pylori.

INTRODUCTION

Chronic gastritis is an inflammatory condition of the gastric mucosa that may include structural alterations of the glandular compartment [1], it's still one of the most prevalent major pandemic illnesses with fatal complications like stomach cancer or peptic ulcers [2]. Studies have reported that *Helicobacter pylori* infection is the main etiologic and pathogenic factor of chronic gastritis worldwide, degree of inflammation and the evolution of this form of chronic gastritis can vary largely depending on bacterial virulence factors, host susceptibility, and environmental conditions [3, 4]. Metaplasia is the replacement of one differentiated cell type with another mature differentiated cell type that is not normally present in a specific tissue, gastric intestinal metaplasia (GIM) is histologically defined as changes in gland structure to resemble intestinal glands and the presence of intestine specific lineage cells such as (goblet cells and Paneth cells) that are not present in the normal stomach, molecularly characterized by expression of intestine-specific transcription factors of the caudal type homeobox (CDX) family, CDX1 and CDX2(13). CDX2 homeodomain protein which is not expressed in normal gastric mucosa is a sensitive

Citation: Zeinab Ali Mohamed, Ibrahim B Elemam, Mohammed Abdelgader Elsheikh, Tibyan Abd Almajed Altaher, Ghanem Mohammed Mahjaf, Mosab Nouraldein Mohammed Hamad (2023). Immunohistochemical Expression of CDX2 as Early Biomarkers for Gastric Intestinal Metaplasia among Sudanese Patients with Chronic Gastritis. *SAR J Pathol Microbiol*, 4(5), 58-64.

marker for intestinal metaplasia and is closely associated with the intestinal type of adenocarcinoma. So their expression in gastric mucosa is strongly associated with intestinal metaplasia and gastric carcinoma [5]. In adult humans, CDX2 acts as a tumor suppressor which is strictly confined to the intestinal tract development and differentiation during embryogenesis so their expression in the upper digestive tract is pro-oncogenic and considered to be a trigger in the progression of gastric intestinal metaplasia [6]. The expression levels of CDX2 were shown to be significantly increased in gastric mucosal metaplastic lesions and were closely related to *H. pylori* infection [7]. The prevalence of IM in the worldwide population was 25% whereas extensive IM was found in 13% [8]. IM was found in 12.7% of 300 cases in various Italian trials with consecutive dyspeptic patients who had *H. pylori* infection and a mean age of 49 years [9].

In Western countries, AG and IM are generally observed in histological examination of random biopsies obtained during endoscopy, whereas in Asian countries including Korea, the presence and extension of AG and IM are frequently observed by endoscopy, the prevalence of endoscopic AG and IM were 40.7% and 12.5%. Age groups of 40 to 59 years and >60 years, male sex, and positive *H. pylori* serology were risk factors for AG [10]. Epidemiological studies have shown that IM is significantly associated with an increased risk of gastric carcinoma approximately 80% of gastric carcinomas occur in the setting of IM and the relative risk for cancer development in the presence of IM is 6.4 [11]. GIM is closely related to the development of intestinal-type gastric cancer, and its formation can increase the risk of gastric cancer more than 10 times [12]. The prevalence of gastric intestinal metaplasia was highest among Hispanic patients (29.5% followed by African American (25.5% and non-Hispanic white patients (13.7%), after we adjusted for age, sex, and smoking, African American and Hispanic race or ethnicity, and *H pylori* infection were associated with an increased risk of gastric intestinal metaplasia Race or ethnicity, was associated with stomach intestine metaplasia in 33.6% of cases due to *H pylori* infection alone, and in 55.5% of cases when acute and chronic gastritis was present. According to upper endoscopy biopsy research, individuals who are Hispanic and African American have a higher risk of developing stomach intestine metaplasia than patients who are non-Hispanic white [13].

MATERIALS AND METHODS

Study area

This study was conducted in River Nile State, at Atbara Medical Complex and El-makNimr University Hospital, the major Hospitals in the State contain histopathology and cytopathology laboratory and Gastro endoscopic department with a high-frequency rate of patients during the period from October 2019 to December 2022.

Study design

This is a descriptive retrospective cross-sectional study aimed to detect caudal-type home box gene CDX2 expression as an early indicator for intestinal metaplasia among Sudanese patients with chronic gastritis.

Study population

Archived Formalin fixed paraffin embedded (FFPE) gastric tissue blocks diagnosed with chronic gastritis were collected during the period from 2013 to 2021.

Materials

Archived formalin-fixed paraffin-embedded (FFPE) gastric tissue blocks obtained from patients previously diagnosed with chronic gastritis were included in this study.

Sample preparation and processing

Formalin-fixed paraffin-embedded blocks of gastric tissue with chronic gastritis were processed from the same tissue block one section (10 µm) was cut and placed in an Eppendorf tube for molecular analysis and (3µm) for histological detection by using Leica rotary microtome (RM 2125), 7 glass slide form each patient were cut and spread in preheated water path Leica (HI 1210) 45 C°, then put in forested end slide and salinized slides (Dako) for IHC. Paraffin sections were partially deparaffinized in the oven (Memmert GmbH+ CO.KG) at 56 ˚C for 30 minutes for initial deparaffinization and adhesion, then sections were stained by Mayer's Hematoxylin and Eosin technique, combine Alcian blue periodic acid Schiff's, Modified Giemsa stain, Methylene blue stain, Warthin-Starry technique, and Immunohistochemical techniques.

Immunohistochemical staining

Paraffin sections 3 um were partially deparaffinized in the oven (memmert GmbH+ CO.KG) at 56 ˚C for 30 minutes, and inserted in xylene for 3 minutes. Slides were rehydrated in descending grades of alcohol 100%, 90%, and then 70% for 5 minutes each. The slides were distilled water washed for five minutes. Antigen retrieval was performed by boiling using a water bath (PT link), slides were placed in the PT tank containing enough Tris buffer (PH 9.0) to cover the section, then the machine was turned on at 20 minutes to start heating from 65 C° until reach 95 C°and then boiled at high temp (95 C°) for20 mints then allow sections to cool to 65 C° [41].

Immunohistochemistry stain of CDX2

Endogenous peroxidase activity was blocked with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10 min, the slides were incubated with (100 – 200 microliter) Rabbit Anti-CDX2 IgG primary antibody (ZYTOMED system) (see appendix4) was concentrated then diluted 1:100 for 20 min at room [temp in a humidity moisture chamber.

The binding of antibodies was then determined by incubating for 20 minutes with poly dextran tagged polymer (Dako - Envision TM Flex kit) following a 3-minute PBS wash. After three rounds of PBS washing, the sections were then stained with 3, 3 diaminobenzidine tetrahydrochloride (DAB) (Dako) to create the distinctive brown stain for the observation of the antibody/enzyme combination for up to five minutes. The slides were rinsed with H2O and counter-stained with hematoxylin for 3 minutes. This was followed by washing with running water (10 min). Sections were dehydrated in ascending grades of alcohol and cleared with xylene, then cover slipped and examined. The protocol is according to the instruction of the manufacturer [41].

Result interpretation of IHC

Detection of more than 5 cells with brown nuclei in one field is considered a positive result. All quality control measures were adopted, and positive and negative slides were used during immunohistochemical staining (Negative control by the omission of primary Ab positive control for CDX2 was colon).

Ethical Considerations

Ethical approval for the study was obtained from the Board of the Faculty of medical laboratories sciences, at University of Shendi. The written informed consent form was obtained from each guardian of the participant as well as from the subject himself before recruitment into the study. All protocols in this study were done according to the Declaration of Helsinki (1964).

RESULTS

A total of 140 archived paraffin-embedded tissue blocks from patients previously diagnosed with chronic gastritis attending Atbara Medical Complex and El-MakNimr University Hospital at River Nile State were enrolled in this study, 80 were females (57.1 %) and 60 were males (42.9%). The patient's ages ranged from (12 to 97) with a mean age of (53.27) years old, the age was categorized into three groups, less than 40 (27) (19.3%), 40- 60 (64) (45.7%), more than 60 (49) (35.0%). The prevalence of gastric intestinal metaplasia among patients with chronic gastritis was (17.9%) as illustrated in (Table 1), there was no statistically significant correlation between gastric intestinal metaplasia (CDX2 expression) with gender and age as the *P.value* was (0.308) and (0.256) respectively as demonstrated in (Table 2&3).

Table 1: Gastric intestinal metaplasia (CDX2 expression) detected by immunohistochemistry

IHC(CDX2)	Frequency	Percentage %
Positive	25	17.9
Negative	115	82.1
Total	**140**	**100%**

Table 2: Correlation of Gastric intestinal metaplasia (CDX2 expression) with gender

CDX2	*Gender*		*Total*	*P.value*
	Male	*Female*		
Positive	13	12	25	0.308
Negative	47	68	115	
Total	**60**	**80**	**140**	

Table 3: Correlation of Gastric intestinal metaplasia (CDX2 expression) with age group

CDX2	Age group			Total	*P.value*
	Less than 40	40-60	More than 60		
Positive	2	14	9	25	0.256
Negative	25	50	40	89	
Total	**27**	**64**	**49**	**140**	

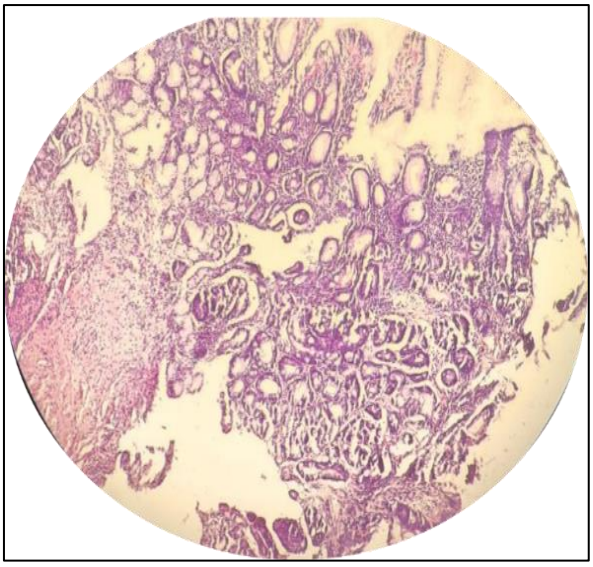

Figure 1: Chronic gastritis: showing chronic inflammatory cellular infiltrate within the lamina propria (H&E X 10)

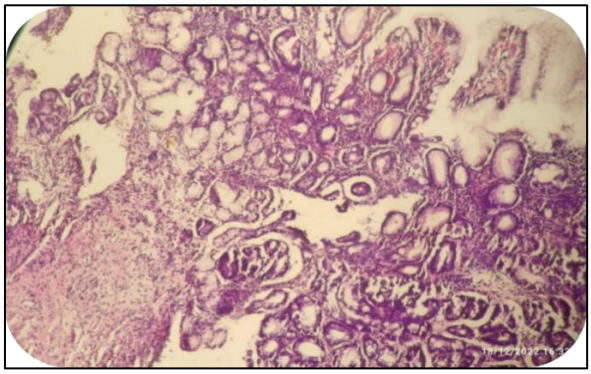

Figure 2: Gastric antral mucosa showing intestinal metaplasia (mucosal goblet cell formation, arrowed). (H&E stain Magnification x 10)

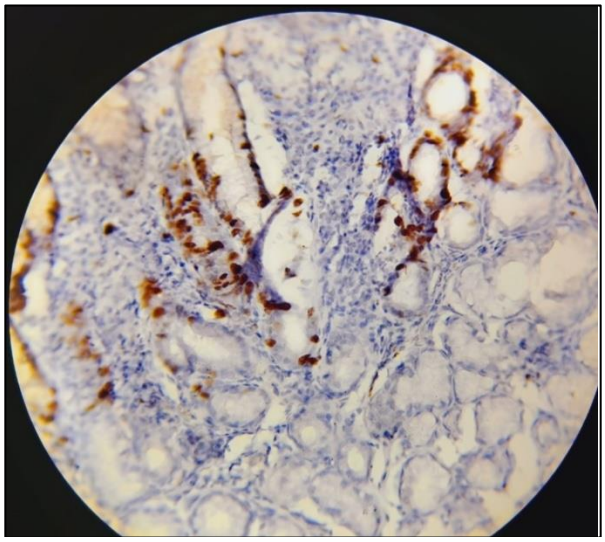

Figure 3: CDX2 positive using immunohistochemistry in chronic gastritis x40

DISCUSSION

The most recent epidemiological data indicated that gastric cancer (GC) remains the fifth most frequent cancer and the four leading cause of cancer-related deaths worldwide. Gastric intestinal metaplasia is a known premalignant condition of the human stomach along the pathway to gastric cancer, at the histological level GIM is characterized by goblet and absorptive cells or columnar non-absorptive cells. GIM are the

histopathologic entities that reflect the ordinal phases during the conversion of chronic gastric inflammation to carcinoma [14]. According to Correa's gastric cancer cascade, GIM is a major risk factor for gastric cancer. It was discovered in 25.3% of patients with dyspepsia and 100% of patients with intestinal-type gastric cancer. Approximately 1 in every 39 GIM patients will get gastric cancer within 20 years [15, 16]. Because *H. pylori* are the most common bacterial infection worldwide, therefore, its early proper diagnosis and subsequent successful eradication represent the cornerstones of gastric cancer prevention. Challenges related to accurate diagnosis lead to a choice that must be based on *H. pylori* virulence, environmental factors, and host genetic characteristics [17, 18]. Bile reflux, dietary habit, alcohol consumption, smoking, increased salt intake, increasing age, male gender, and family history of gastric cancer is established factors associated with IM progression [19, 20]. The main aim of the current study investigated the early detection of gastric intestinal metaplasia among Sudanese patients diagnosed with chronic gastritis by using CDX2 expression protein as a sensitive marker for gastric intestinal metaplasia, and evaluate the different conventional techniques in the detection of GIM and *H. pylori.* Regarding the gender distribution, females were more affected by chronic gastritis rather than male, this finding was in agreement with many authors in Sudan, Sied and Hamza [21].

Ali *et al.*, [22] Nogdalla *et al.*, [23] and Mohammed Almosfa Kamal *et al* [24] also in Saudi Arabia by Alkhamiss *et al* [25] Mexico Atrisco-Morales *et al* [26] South Africa by Idowu *et al* [27] and disagree with study in Iraq by Aziz *et al* [28] in India Pandya *et al* [29] and Shetty *et al* [30]. The important factors affecting the incidence of chronic gastritis due to unhealthy eating habits, such as not eating on time, preferring hot food, and often overeating may cause gastrointestinal dysfunction, gastric acid secretion disorder, and increase the risk of chronic gastritis [31]. In Sudan, women culturally do most of the housekeeping, cooking, and food preparation exposing them to injury also and eating habits such as intermittent meals, irregular diet, eating hot and spicy food, and pickled vegetables that might be exposed them to infection rather than the male with chronic gastritis also women lived in stressful setups, had less time for taking rest, wakening up early from bed, and later going to bed for sleeping, other factors lead to this result females are more likely to visit health facilities than males. Regarding age the current study reveals the highest proportion of chronic gastritis (45.7 %) was found in middle and elder patients aged (40to 60) years, this finding was near to the Study done in Sudan by Ali *et al* [22], another study in Iraq Shehab *et al* that reported the highest incidence of gastritis was observed to occur between the third and fifth decades [32] and disagree with a study done in Guinea by Cheng *et al*, that reported the highest proportion of patients aged 18 to 30 [31], the discrepancy of results might be as a result of the methodologies, sampling strategies employed, sample size. Generally, the prevalence of chronic gastritis increased with age suggesting that age can be a crucial factor for having gastritis due to thinning stomach lining with aging because aging is related to a decreased rate of gastric epithelial cell turnover and a decreased ability to repair the gastric mucosa due to decreased levels of prostaglandin in the gastric mucosa [32].

Metaplasia of the stomach's intestines is a critical stage in the growth of stomach cancer. Few and limited data are available regarding the prevalence of gastric intestinal metaplasia in Sudan, the current study spots light on the prevalence of gastric intestinal metaplasia among Sudanese immunohistochemistry is used in patients with chronic gastritis (CDX2 expression). The CDX2 gene encodes a member of the homeobox caudal protein family, CDX2, which is likely involved in intestinal epithelial differentiation and proliferation [33]. It could predict the behavior of chronic gastritis disease. In the current study CDX2expresstion was (17.9%), this prevalence is enough to get attention about this serious step to gastric cancer through more biological studies, using accurate biomarkers, and medical intervention including early detection of *H. Pylori* as the main causation of gastric intestinal metaplasia these findings were in agreement with many previous studies done in Thailand by Chitapanarux *et al* 19.3% [34] by Eriksson *et al* (19%) [35]. A studywas done in Nepal by Pradhan *et al* (12.2%) of gastric intestinal metaplasia [36]. In Morocco by El Gui *et al* [37]. In China by Zhang *et al* (13.2%) [38]. The prevalence of GIM in the general population is known to vary around the globe because most patients with intestinal metaplasia remain asymptomatic unless cobalamin deficiency occurs secondary to loss of glands (that produce intrinsic factor and acid) loss of intrinsic factors and the decrease of gastric acid production lead to cobalamin and folic acid malabsorption, the endoscopic finding of IM is observed as a mucosal nodular pattern, usually occurring after the occurrence of the AG [20].

Due to the asymptomatic nature of the lesion and its dependence on *H. pylori* status, particularly the prevalence of *H. pylori* infection and the many diagnostic techniques employed in research, it is still difficult to determine the prevalence of gastric IM in the general population. Variationsin sample size, and, molecular markers, all these factors make the prevalence of GIM challenging and need more comprehensive studies. Our result found that CDX2 expression has no statistical correlation with age and gender with *P.value* (0.697,0.308) respectively this result was agreeing with the Study done in China by Zhang *et al.*, [38] CDX2 expression not correlated with clinical parameters, such as age, gender (*P.value* 0.326) and (*P.value* 1.000) respectively [38]. And disagree

with another study in Egypt done by Helal *et al* [39] reported that CDX2 expression was significantly associated with the male sex (*P.value*> 0.001), regardless of gender suggesting that sex-specific characteristics, such as alcohol, smoking, and eating habits, including salty or spicy foods, are related to the development of atrophy, IM in addition to *H pylori* infection economic and hygienic conditions [40]. This variation in sex and age may be due to the selection strategy of patients' samples only reflecting the presence of patients at the time of collection in our study explained based on the fact that most of the patients who underwent gastro endoscopy are adult age who suffering from an infection for long period, lead to deciding on the clinician to take biopsy this may be the reason behind the large scale of our study population over than 40 years old, also most children have eradicated from *H. pylori* infection by responding quickly to treatment. The prevalence of gastric intestinal metaplasia among chronic gastritis patients is 17.9%.

CONCLUSION

The development of gastric intestinal metaplasia as a premalignant condition of gastric carcinoma is important to detect this change at an early point to prevent and control gastric cancer.

Sources of Funding

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors.

Conflict of Interest

Authors have declared that no competing interests exist.

REFRENCES

1. Rugge, M., & Genta, R. M. (2005). Staging and grading of chronic gastritis. *Human pathology*, *36*(3), 228-233.
2. Sipponen, P., & Maaroos, H. I. (2015). Chronic gastritis. *Scandinavian journal of gastroenterology*, *50* (6), 657-667.
3. Varbanova, M., Frauenschläger, K., & Malfertheiner, P. (2014). Chronic gastritis–an update. *Best Practice & Research Clinical Gastroenterology*, *28*(6),1031-1042.
4. Fitzmaurice, C., Dicker, D., Pain, A., Hamavid, H., Moradi-Lakeh, M., MacIntyre, M. F., ... & Global Burden of Disease Cancer Collaboration. (2015). The global burden of cancer 2013. *JAMA oncology*, *1*(4), 505-527.
5. Sandhya, S., Mathew, C. F., & Jisha, K. T. (2015). Expression of CDX2 protein in gastric mucosa with intestinal metaplasia and gastric carcinoma. *Indian J. Pathol. & Oncol*, *2*(3), 150-157.
6. Zhou, H., Ni, Z., Li, T., Su, L., Zhang, L., Liu, N., & Shi, Y. (2018). Activation of FXR promotes intestinal metaplasia of gastric cells via SHP-dependent upregulation of the expression of CDX2. *Oncology Letters*, *15*(5), 7617-7624.
7. Guo, R. J., Suh, E. R., & Lynch, J. P. (2004). The role of Cdx proteins in intestinal development and cancer. *Cancer biology & therapy*, *3*(7), 593-601.
8. de Vries, A. C., van Grieken, N. C., Looman, C. W., Casparie, M. K., de Vries, E., Meijer, G. A., & Kuipers, E. J. (2008). Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology*, *134*(4), 945-952.
9. Vaira, D., Zullo, A., Vakil, N., Gatta, L., Ricci, C., Perna, F., ... & Morini, S. (2007). Sequential therapy versus standard triple-drug therapy for Helicobacter pylori eradication: a randomized trial. *Annals of internal medicine*, *146*(8), 556-563.
10. Joo, Y. E., Park, H. K., Myung, D. S., Baik, G. H., Shin, J. E., Seo, G. S., ... & Kim, N. (2013). Prevalence and risk factors of atrophic gastritis and intestinal metaplasia: a nationwide multicenter prospective study in Korea. *Gut and liver*, *7*(3), 303.
11. Almeida, R., Almeida, J., Shoshkes, M., Mendes, N., Mesquita, P., Silva, E., ... & David, L. (2005). OCT-1 is over-expressed in intestinal metaplasia and intestinal gastric carcinomas and binds to, but does not transactivate, CDX2 in gastric cells. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *207*(4), 396-401.
12. Li, Q., Zhu, Y., Liu, J., Yu, X., Chen, M., Dong, N., ... & Yuan, Y. (2017). HpSlyD inducing CDX2 and VIL1 expression mediated through TCTP protein may contribute to intestinal metaplasia in the stomach. *Scientific Reports*, *7*(1), 2278.
13. Nguyen, T. H., Tan, M. C., Liu, Y., Rugge, M., Thrift, A. P., & El-Serag, H. B. (2021). Prevalence of gastric intestinal metaplasia in a multiethnic US veterans population. *Clinical Gastroenterology and Hepatology*, *19*(2), 269-276.
14. Kutluana, U., & Kilciler, A. G. (2019). Is there a possible relationship between gastric intestinal metaplasia and systemic arterial stiffness. *Rev Esp Enferm Dig*, *111*(7), 500-506.
15. Shao, L., Li, P., Ye, J., Chen, J., Han, Y., Cai, J., & Lu, X. (2018). Risk of gastric cancer among patients with gastric intestinal metaplasia. *International journal of cancer*, *143*(7), 1671-1677.
16. Wei, N., Zhou, M., Lei, S., Zhong, Z., & Shi, R. (2021). A meta-analysis and systematic review on subtypes of gastric intestinal metaplasia and neoplasia risk. *Cancer Cell International*, *21*(1), 1-9.
17. Loharamtaweethong, K., & Puripat, N. (2020). Comparison of immunohistochemistry and conventional stains for Helicobacter pylori detection in gastric biopsies of patients receiving proton pump inhibitors. *Journal of Health Science and Medical Research*, *38*(4), 321-330.
18. Mărginean, C. O., Meliț, L. E., & Săsăran, M. O. (2022). Traditional and Modern Diagnostic Approaches in Diagnosing Pediatric Helicobacter pylori Infection. *Children*, *9*(7), 994.
19. Compare, D., Rocco, A., & Nardone, G. J. E. R. M. P. S. (2010). Risk factors in gastric cancer. *European Review for Medical & Pharmacological Sciences*, *14*(4).
20. Malik, T. H., Sayahan, M. Y., Al Ahmed, H. A., & Hong, X. (2017). Gastric intestinal metaplasia: an i

ntermediate precancerous lesion in the cascade of g astric carcinogenesis. *J Coll Physicians Surg Pak*, *2 7*(3), 166-172.
21. Sied, K. M., & Hamza, A. A. (2015). Reliability of histological staining methods in the detection of He licobacter pylori: comparison of three staining met hods. *Int J Adv Res*, *3*, 1163-9.
22. Ali, E. T., Fadul, T. M., Nasr, M. A., Siddig, E. E., Mohamed, N. S., & Edris, A. M. M. (2018). Comp arison between immunohistochemical marker and c onventional histochemical stains in detecting Helic obacter pylori. *EC Microbiol*, *14*(8), 430-437.
23. Nogdalla, A.M.A. (2001). Detection of Helicobacter pylori CagA Gene in Gastric Lesions among Sudanese Gastric Disorder Patients: *Sudan University of Science & Technology*.
24. Yousif Elemam, I. B., Ibrahim, A. A., Mohamadein , A. O., Abdel Rahim, S., AlmosfaKamal, M., & A bugooda Alobaid, A. E. A. A. (2017). Detection of Intestinal Metaplasia among Sudanese Patients wit h Chronic Gastritis by Using Combined Alcian Blu ePAS Technique. *International Journal of Current Research in Medical Sciences*, 3(6), 808.
25. Alkhamiss, A. S. (2020). Evaluation of better staining method among hematoxylin and eosin, giemsa and per iodic acid schiff-alcian blue for the detection of helico bacter pylori in gastric biopsies. *The Malaysian Journ al of Medical Sciences: MJMS*, *27*(5), 53.
26. Atrisco-Morales, J., Martínez-Santos, V. I., Román-R omán, A., Alarcón-Millán, J., De Sampedro-Reyes, J., Cruz-del Carmen, I., ... & Fernández-Tilapa, G. (201 8). vacA s1m1 genotype and cagA EPIYA-ABC patte rn are predominant among Helicobacter pylori strains isolated from Mexican patients with chronic gastritis. *Journal of Medical Microbiology*, *67*(3), 314.
27. Idowu, A., Mzukwa, A., Harrison, U., Palamides, P., Haas, R., Mbao, M., ... & Njom, H. (2019). Detection of Helicobacter pylori and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospit al, South Africa. *BMC gastroenterology*, *19*(1), 1-10.
28. Aziz, Z. W., Saleem, S. H., & Al-Nuaimy, H. A. (202 0). Helicobacter pylori in gastric biopsy: a histochemi cal and immunohistochemical assessment. *Annals of t he College of Medicine, Mosul*, *41*(2), 139-147.
29. Pandya, H. B., Patel, J. S., Agravat, H. H., Patel, S. B., & Thakkar, M. C. (2013). Identification of Heli cobacter pylori by different conventional staining t echniques and its comparison with polymerase chai n reaction. *Saudi Med J*, *34*(9), 942-948.
30. Shetty, V., Lingadakai, R., Pai, G. C., & Ballal, M. (2021). Profile of Helicobacter pylori cagA & vacA genotypes and its association with the spectrum of gastroduodenal disease. *Indian Journal of Medical Microbiology*, *39*(4), 495-499.
31. Cheng, Y., Keita, M., & Si, X. B. (2021). Investigat ion on Risk Factors of Chronic Gastritis in the Pop ulation of Conakry in Guinea. *Indian Journal of Ph armaceutical Sciences*, *83*, 1-5.
32. Shehab, M. J., Abdul-Hassan, I. A., & Mahdi, B. M . (2022). Tumor necrosis factor alpha gene at two p osition as related with gastritis incidence in iraqi pa tients. *Biochemical & Cellular Archives*, *22*(1).
33. Sardar, A. A., Jalal, J. A., & Ameen, K. S. H. (2022). Immunohistochemical Expression of CDX2 in Gastric Carcinoma. *Iranian Journal of Pathology*, *17*(2), 143.
34. Chitapanarux, T., Jesadaporn, P., Chitapanarux, N., & Lertprasertsuke, N. (2021). Chronic gastritis acc ording to age and Helicobacter pylori in Thailand: histopathological patterns. *Scandinavian Journal of Gastroenterology*, *56*(3), 228-233.
35. Eriksson, N. K., Kärkkäinen, P. A., Färkkilä, M. A. , & Arkkila, P. E. (2008). Prevalence and distributi on of gastric intestinal metaplasia and its subtypes. *Digestive and Liver Disease*, *40*(5), 355-360.
36. Chalise, S., Ghimire, R., & Pradhan, S. B. (2020). Pre valence of subtypes of gastric intestinal metaplasia an d its relationship with Helicobacter pylori infection. *J ournal of Pathology of Nepal*, *10*(1), 1650-1653.
37. El Gui, R., Hechlaf, H., Ed-Day, S., Akhouayri, O., Boulbaroud, S., & Azzaoui, F. Z. (2021). Histo-epi demiological aspect of Helicobacter pylori chronic gastritis in a Moroccan population. In *E3S Web of Conferences* (Vol. 319, p. 01027). EDP Sciences.
38. Zhang, Y., Wang, H., Bi, C., Xiao, Y., & Liu, Z. (2 016). Expression of CDX2 in gastric cardia adenoc arcinoma and its correlation with H. pylori and cell proliferation. *Oncotarget*, *7*(34), 54973.
39. Helal, N., Omran, Z., Aboushousha, T., Youssef, M ., Badawy, A., Aziz, A. A., ... & Moussa, M. M. (2 020). Predictive value of CDX2 and SOX2 in chron ic gastritis and intestinal-type gastric cancer. *Open Access Macedonian Journal of Medical Sciences*, *8* (A), 947-955.
40. Kwon, Y. J., Kim, N., Baek, S. M., Lee, H. S., Lee, J., Hwang, Y. J., ... & Lee, D. H. (2019). The prev alence of histologic atrophy and intestinal metaplas ia in the corpus has decreased over 15 years in fem ales in the Korean population. *Helicobacter*, *24*(3), e12579.
41. Bancroft, J.D., & Gamble, M. (2008). Theory and practice of histological techniques: Elsevier health sciences. 6 Ed.410.