ISSN: 0067-2904

Comparing the Expression of *CDX2* & SATB2 in Samples of Iraqi Patients with Colorectal Cancer

Rasha Abbas Azeez[1*], Suha Abdul-Khaliq Al-Jowari[2], Hayder Jamal Mahmood.[3]
[1] Department of Basic Science, College of Dentistry, University of Baghdad, Baghdad, Iraq
[2]Department of Biology, Colloge of Science, University of Baghdad, Baghdad, Iraq
3 Endoscopy Department at the Gastroenterology and Hepatology Hospital Medical city Department, Baghdad, Iraq

Received: 26/5/2023 Accepted: 9/10/2023 Published: 30/12/2024

Abstract

The purpose of this study was to assess the diagnostic value of the immunohistochemical expression of Special AT-rich sequence binding protein (SATB2) alone and in combination with the caudal type homeobox 2 (*CDX2*) in colorectal cancers (CRCs) in Iraqi patients. This study's samples collection for tissue and practical work spanned twelve months, 20 December 2021 to 20 December 2022. A total of 80 male and female participants (41 CRC, 19 severe colitis and 20 controls) were examined. For each case, at least two 4-µm-thick tissue sections were cut for immunohistochemical staining with *CDX2* and SATB2 immunohistochemical markers. According to the current study results all samples of CRC patients showed positive expression for STAB2 and *CDX* proteins with significantly higher ($p< 0.001$) frequencies of expression recorded in score 3, 78.05% and 99.9% respectively, in comparison to other study groups. A positive correlation between the two studied markers was found and both could be used as diagnostic markers for CRC.

Keywords: Colorectal cancers, immunohistochemical study, expression, SATB2, *CDX2*.

مقارنة تعبير *CDX2* و SATB2 في عينة من المرضى العراقيين المصابين بسرطان القولون– المستقيم

رشا عباس عزيز[1]، سهى عبد الخالق الجواري[2]، حيدر جمال محمود[3]
[1] فرع العلوم الأساسية، كلية طب الاسنان، جامعة بغداد، بغداد، العراق.
[2] قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.
[3] قسم النواظير في مستشفى أمراض الجهاز الهضمي والكبد التعليمي مجمع المدينة الطبية، بغداد، العراق

الخلاصة

كان الغرض من هذه الدراسة هو تقييم القيمة التشخيصية للتعبير الكيمو نسيجي المناعي لـ SATB2 وحده وبالاشتراك مع *CDX2* في سرطان القولون والمستقيم (CRCs) للمرضى العراقيين .استغرقت مدة جمع عينات هذه الدراسة للأنسجة وللعمل المختبري اثني عشرشهرا،من ديسمبر 2021 إلى ديسمبر 2022 بلغ العدد الإجمالي للمشاركين 80 مشاركا من الذكور والإناث(مجموعة الورم الخبيث وعددها 41، مجموعة التهاب القولون الشديد تتكون من 19 عينة،مجموعة السيطرة ضمت 20 عينة) لكل حالة تم قطع قسمين

*Email: rasha.abbas@codental.uobaghdad.edu.iq

على الأقل من الأنسجة بسمك 4 مايكروميتر، تم التصبيغ الكيمو نسيجي المناعي باستخدام معلمات *CDX2* وSATB2 وفقا لنتائج الدراسة الحالية ، أظهرت جميع عينات مرضى CRC تعبيرا إيجابيا لبروتينات STAB2 و *CDX2* مع ارتفاع معنوي ($p< 0.001$) ومعدلات تعبير عالية في الدرجة 3وبقيم 78.05٪ و 99.9٪ على التوالي ، مقارنة بمجموعات الدراسة الأخرى. تم العثور على علاقة إيجابية بين المعلمين المدروسين ويمكن استخدام كلاهما كمعلمات تشخيصية لسرطان القولون والمستقيم.

1. Introduction

The prevalence of colorectal cancer (CRC), which has overtaken all other cancers as the second leading cause of death worldwide, is also gradually rising in the developing countries [1, 2]. According to GLOBOCAN 2018 data, rectum cancer is the eighth most prevalent type of cancer worldwide Whereas CRC is the third most common cancer type to be diagnosed worldwide, making up 11% of all cancer diagnoses [3, 4, 5]. Between 2000 and 2019, the proportion of all CRC cases to all other cancers in Iraq increased from 3.69% to 6.5%. And according to an Annual Percentage Changes (APC) there was an increase of 3.54% [6, 7], the CRC mortality proportion grew from 1.25 to 1.77 per 100 000 persons between 2010 and 2019 respectively. The development of CRC occurs in the glandular epithelial cells of the large intestine, also known as colorectal adenocarcinoma. Cancer arises when specific epithelial cells undergo a series of epigenetic or genetic changes [8, 9]. Due to their abnormally high replication and survival rates, the hyper-proliferative cells generate benign adenomas that can later develop into cancer and spread over decades [10].

The special AT-rich sequence binding-protein (SATB2) is a 733 amino acid long DNA-binding protein that specifically binds to nuclear matrix attachment regions of DNA. It is involved in the regulation of transcription and chromatin remodeling, and exhibits an amazing level of evolutionary conservation, with only three amino acids differing between mouse and human. Some recent studies evaluated antibodies for SATB2 detection in over1,800 cases of CRC and over 600 cases of various other cancers [11, 12, 13]. As SATB2 is expressed voluntarily in the lower gastrointestinal tract, it is possible to use it as a diagnostic indicator for colorectal cancer. This possible diagnostic biomarker has been studied in order to better understand colorectal carcinoma and other cancer types [14, 15].

A particular intestinal transcription factor expressed in the nucleus of intestinal epithelial cells is the Caudal-type homeobox transcription factor 2 *CDX2* gene [16]. The embryonic development and differentiation of the gut are influenced by the *CDX2* gene. As *CDX2* is involved in cell proliferation and differentiation, it is typically regarded as a useful single marker. Therefore, it is important to think about how *CDX2* expression may affect prognosis. It's interesting to note that *CDX2* can be expressed in various cancerous tumors including bladder, ovarian, lung and biliary carcinomas [17]. The expression levels of *CDX2* in CRCs range from 26.7% to 100%. Despite being a particular marker, *CDX2* is not always positive, and the expression of this marker might vary depending on the type of tumor and the evaluation method. The down regulation of *CDX2* expression may be related to loss of differentiation. Previous research has shown a link between poor survival and a decline of *CDX2* expression [18, 19, 20].

The purpose of this study was to evaluate the diagnostic value of immunohistochemical expression of SATB2 alone and in combination with *CDX2* in colorectal cancers (CRCs) in Iraqi patients.

Material and Methods

Ethical Approval

The Ethics Committee of the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, approved this research. Committee number CSEC/1121/0076 dated 15 November 2021, as did the Iraqi Ministry of Health and Environment, Medical City Department of Gastroenterology Hospital in their record to facilitate task number 46736 on 14 December 2021. The objective of the study was to be prospective. There was a total of 80 male and female participants with an average age ranging from 24 to 77 years old. All 80 cases (41 CRC, 19 severe colitis and 20 controls) were retrieved at Medical City Department of Gastroenterology Hospital.

Study Design and Subjects

This study's samples collection for tissue and practical work spanned twelve months December 2021 to December 2022. Each individual who participated in the study provided a signed, written consent form. Following surgical or endoscopic resection, colorectal cancer was classified into distinct pathological categories. The histological types were classified based on the International Classification of Diseases [ICD-0 (8140/3), ICD-11 (2B90.Y & XH74S1). CRC was diagnosed according to the ninth edition of the American Joint Committee on Cancer/Tumor [21, 22].

Immunohistochemical Staining

The antigen on formalin fixed, paraffin embedded (FFPE) tissues was identified sing antibodies. The antigen and antibody complex were visualized using an enzyme-coupled secondary antibody (HRP/AP) that binds specifically to the primary antibody, two primary antibodies, manufactured by PathnSitu in USA were employed in the study (*CDX2* clone: EP25 and SATB2 clone: EP281, Rabbit Monoclonal Antibody). This complex is visualized by the enzymatic activation of the chromogen which results in a visible reaction production of the antigenic site [23], and each tissue block is then enrolled in the immunohistochemistry technique. At least two tissue sections with a thickness of 4 micrometers were cut from every induvial patient and put on positively charged slides manufactured by DAKO in Denmark. These slides were then stained simultaneously using immunohistochemical markers (*CDX2* and SATB2) made by PathnSitu biotech. in the USA. To properly prepare histological sections it was necessary to adhere to the established protocols [24].

Semi-quantitative evaluation of *CDX2* and SATB2 expressions was performed by dividing each group into four scores (0, 1, 2, 3) which was based on the percentage of tumor cells with nuclear staining that was estimated by visual inspection by the pathologist. A histochemical scoring (H-score) for SATB2 and *CDX2*, assessment was performed by incorporating both the staining intensity (i) and a percentage of stained cells at each intensity level (Pi). The i values were indicated as 0 (negative no evidence of staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The Pi values varied from 0% to 100%. The final H-score was derived from the sum of i multiplied by Pi as the equation 1 below [25, 26, 27]. Brown positive staining with nuclei (colored chromogens) for SATB2 and *CDX2* results were defined as nuclear staining in tumor cells, regardless of either staining intensity or the proportion of tumor cells with nuclear staining.

$$\text{H-score} = (0 \times P_0) + (1 \times P_1) + (2 \times P_2) + (3 \times P_3) \quad \text{---1}$$

Statistical Analysis

Data description, analysis and presentation were performed using Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA) to characterize the relationship between variables. One Way Analysis of Variance (ANOVA) was applied to compare the difference between k independent groups with using Hochberg GT2 and Games-Howell test and Kendall's tau test. A p value less than 0.05 (p<0.05) was considred statistically significant.

Results and Discussion

Study Group Characteristics

The 80 participants, aged between 24 to 77 years, were included, 41 CRC patients, 19 patients with colitis-associated carcinoma, and 20 healthy subjects as the control group. Furthermore, there were 39 males and 41 females among study groups.

Colon Tissues in patient with Colorectal Cancer compared to healthy individual

In the current study , Figure 1 displays the invasive layer of cancer cells in the colon, indicating malignant glandular tissue without goblet cells. In Figure 2, a cross-section of normal colon tissue is illustrated, presenting straight tubular glands and muscle.

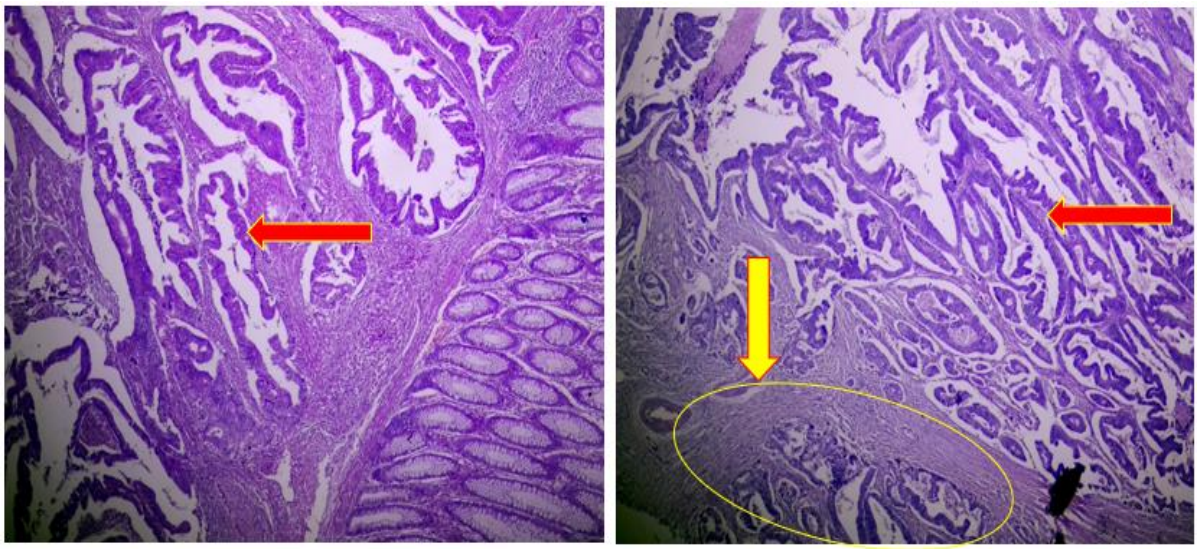

Figure 1: Cross section in colon tissue (colorectal cancer), showing invasive malignant cells in layers well differentiated, loss of goblet cell, red arrow pointing malignant intestinal gland, and yellow arrow pointing invasion H&E stain, X10.

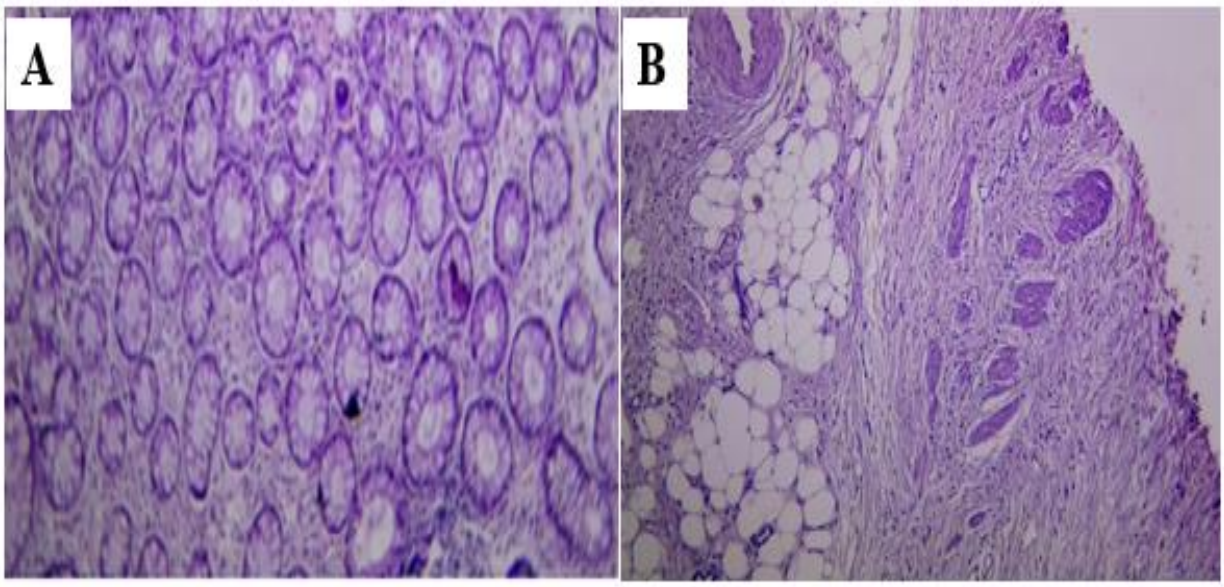

Figure 2: Cross section A in normal colon tissue showing straight tubular gland. H& E stain, 40X, cross section B in normal colon tissue showing muscle. H& E stain, 10X.

SATB2 Expression:

Figure 3 and Table 1 illustrate the varying degrees of brown staining of the nucleus of SATB2 protein expression in the tissue of all groups admitted for the study. The percentage of expression level was divided into four scores (0, 1, 2, 3) for each group. According to the pathologist, they had differing degrees of expression: negative staining, weak, moderate and strong intensity. This was in reference to the colorectal cancer group: low expression of tumor tissue in the examined group of the CRCS in score 1 and high positivity of expression in score 3; low expression 5.26% of colitis tissue in score 3 and high positivity 52.63% in score 1 and no expression in 60% of the control group.

According to the available data, score 3 of strong severity had the highest rate of protein expression (78.05%), followed by 17.07% in score 2 of moderate intensity, and then score 1 of weak intensity In CRC patients (4.88%) (Table 1). All samples of CRC patients (100%) showed positive expression for STAB2 protein with significantly higher ($p< 0.001$) frequency of expression recorded in score 3 in comparison to other study groups.

Table1: Immunohistochemical expression of STAB2 in study groups.

Scores	Studied Groups								Total	
	Colitis		Tumor		Control					
	N	%	N	%	N	%	Fisher exact	P value	N	%
0	1	5.26	0	.00	12	60.00			13	16.25
1	10	52.63	2	4.88	5	25.00			17	21.25
2	7	36.84	7	17.07	3	15.00	73.396	< 0.001	17	21.25
3	1	5.26	32	78.05	0	0.00			33	41.25

*Highly Significant difference (p<0.001),0: No expression 1: Weak 2: Moderate 3: Strong.

In accordance with Ma *et al.* [28], when examined 84% of colorectal adenocarcinomas were SATB2 positive in staining. Similarly, Iwaya *et al.* [29] observed that SATB2 positivity occurred in at least 80% of colorectal adenocarcinomas. Eberhard *et al*. [30] suggested that IHC-based detection of SATB2 was equally sensitive to CRC as *CDX2*, and CDH17, and exhibited good specificity for primary and metastatic lesions of CRC both in animal model of CRC and a prospective patient cohort found that reduced expression of SATB2 was linked to metastasis and a poor prognosis. Most CRCs revealed a widespread SATB2 expression, with nuclear staining in ≤ 90% of tumor cells detected in 65% (679/1039) of cases, and 6% showing a complete lack of SATB2. Its absence helped doctors pinpoint high-risk individuals with more aggressive forms of CRC. SATB2 has been shown to be a more reliable immunohistochemistry indicator in terms of prognosis [31].

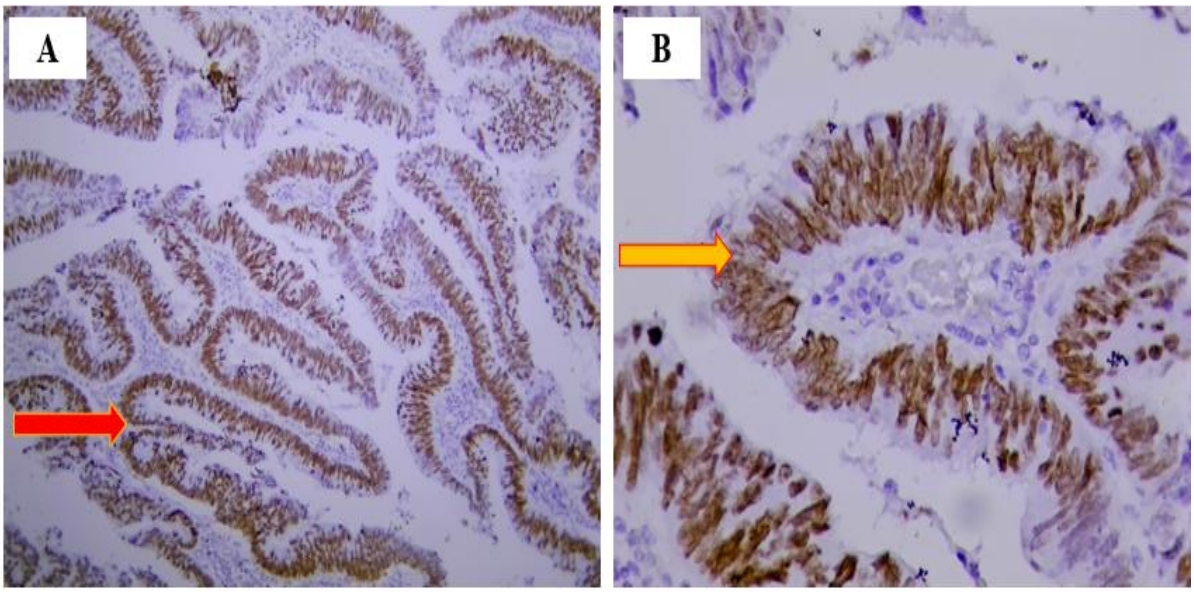

Figure 3: Positive SATB2 (strong intensity) expression in colorectal cancer (A & B), red arrow pointing crypts of Lieberkühn or intestinal gland malignant change and orange arrow pointing Nucleus (original magnification X20, X40).

Consistent with previous research, we found that SATB2 expression was higher in the tumor group than in the control group by a ratio of 78.05%. Furthermore, the results of the current study also agree with Liu *et al*.'s [32] assessment, that 73.85% (96/130) of the 130 cases of colon cancer showed positive SATB2 expression in well-moderately differentiated colon adenocarcinoma. The expression rate of SATB2 was substantially greater than in poorly differentiated adenocarcinoma and it was also significantly higher *($p < 0.001$)* in cases without tumor deposits than in cases with tumor deposits. The percentage of ovarian metastases with positive SATB2 expression was 81.58% (31/38). Other tumors were not found to express SATB2 in a positive manner.

Based on De Michele *et al.* [14], 87% of CRCs tested positive for SATB2. The difference between CRC and lung and pancreatobiliary adenocarcinomas (ADCAs) is where it is most helpful. According to Elnady *et al.* [12] 10% was the best SATB2 cut-off value for differentiating between colonic and non-colonic origin. CK20 was less sensitive and less specific than SATB2. SATB2 may be a reliable diagnostic marker for both primary and metastatic CRC [15]. SATB2 is overexpressed in colorectal cancer and its elevated expression

is indicative of a favorable prognosis [33]. There was similarity between findings of the present study and those of previous research suggest that SATB2 is a diagnostic marker for colorectal cancer.

***CDX2* Expression:**

CRC patients demonstrated an increase in nuclear *CDX2* protein expression. Ninety nine percent of the samples were statistically evenly distributed across three scores (1, 2, 3), indicating that the results of *CDX2* gene protein expression were present in the tissue of all study-approved groups. They varied from weak to moderate to strong. Regarding the group with colorectal cancer, 78.9% of results expressed the protein, and its intensity was measured (1, 2, 3). Seventy-five percent of the normal colon tissue samples included in the analysis lacked gene expression. Table 2 and Figures 4 and 5 demonstrate that there were statistically significant differences *(p <0.001)* between all study groups and the control group of tissue samples.

Table 2: Immunohistochemical expression of CDX2 in study groups.

Scores	Studied Groups								Total	
	Colitis		Tumor		Control					
	N	%	N	%	N	%	Fisher exact	P-value	N	%
0	4	21.05	0	.00	15	75.00			19	23.75
1	4	21.05	3	7.31	4	20.00			11	13.75
2	6	31.58	5	12.19	1	5.00			12	15
3	5	26.31	33	80.48	0	.00	66.768	< 0.001	38	47. 5

***Highly Significant difference (*p*<0.001),**0: No expression 1: Weak 2: Moderate 3: Stron

CDX2 expression, which is more diffusely positive in colorectal carcinomas than in ovarian mucinous adenocarcinoma (POMA), may be the best marker for differentiating colonic carcinoma from primary ovarian mucinous tumor [34]. As reported by Saad *et al.* [35], immunohistochemical studies have revealed that *CDX2* is a specific and sensitive marker for gastrointestinal adenocarcinoma, particularly colorectal adenocarcinoma. A singular marker with diagnostic and therapeutic prognostic capabilities would be very beneficial from a practical standpoint. According to the current paradigm, *CDX2* is a primary "control gene" for intestinal epithelial differentiation [19]. In addition, the absence of *CDX2* expression has been associated with an increase in mortality [20]. Consequently, the expression rates of metastatic CRCs were 95% higher than those of primary CRCs. *CDX2* may therefore be a valuable marker for differentiating metastatic CRCs [17]. The study results support the use of *CDX2* immunohistochemistry as a marker of intestinal cellular differentiation, even in the malignant state.

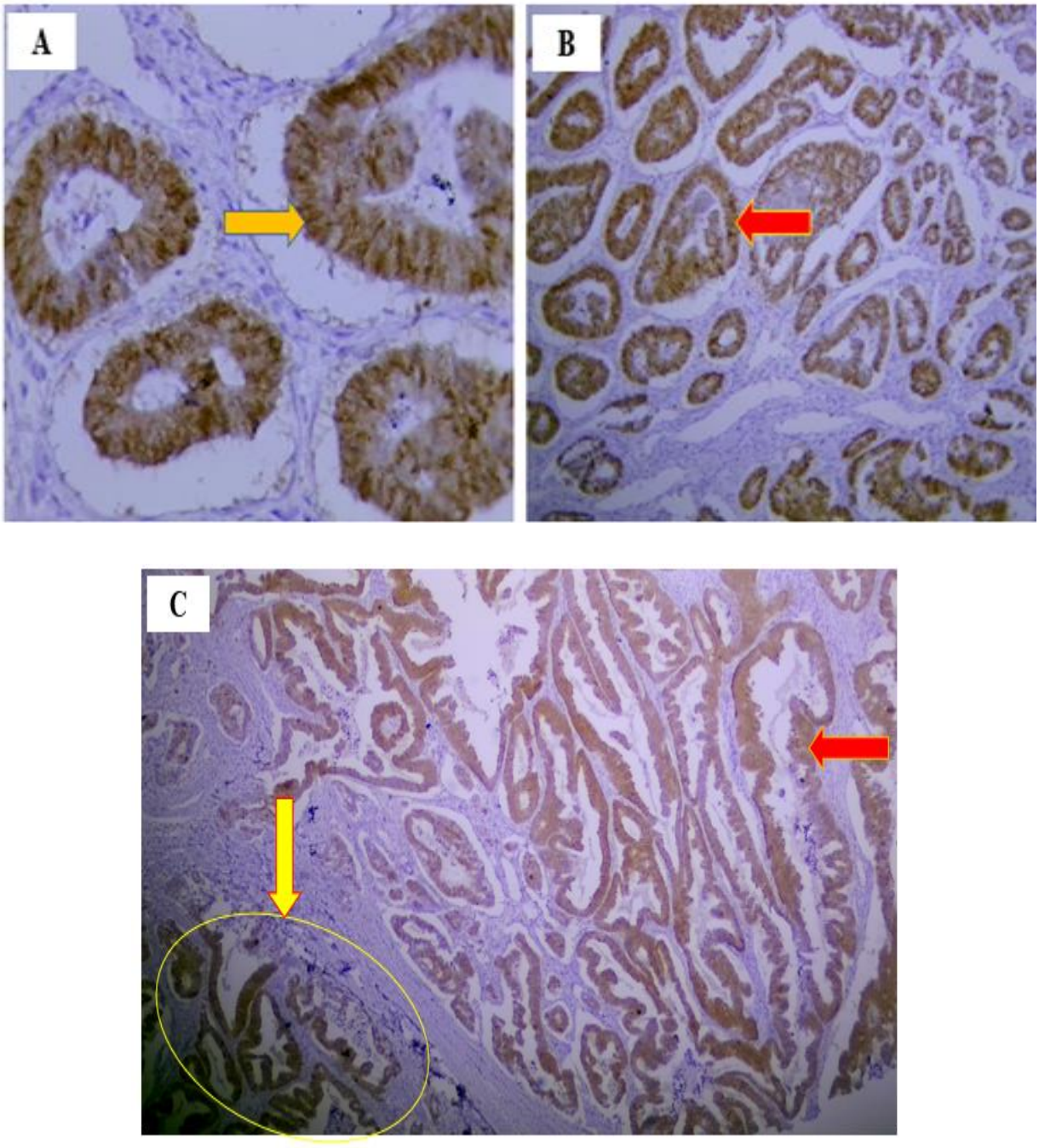

Figure 4: Positive CDX2 (strong intensity) expression in colorectal cancer (A, B & C), Red arrow pointing malignant intestinal gland, orange arrow pointing Nucleus and yellow arrow pointing invasion (X10, X40).

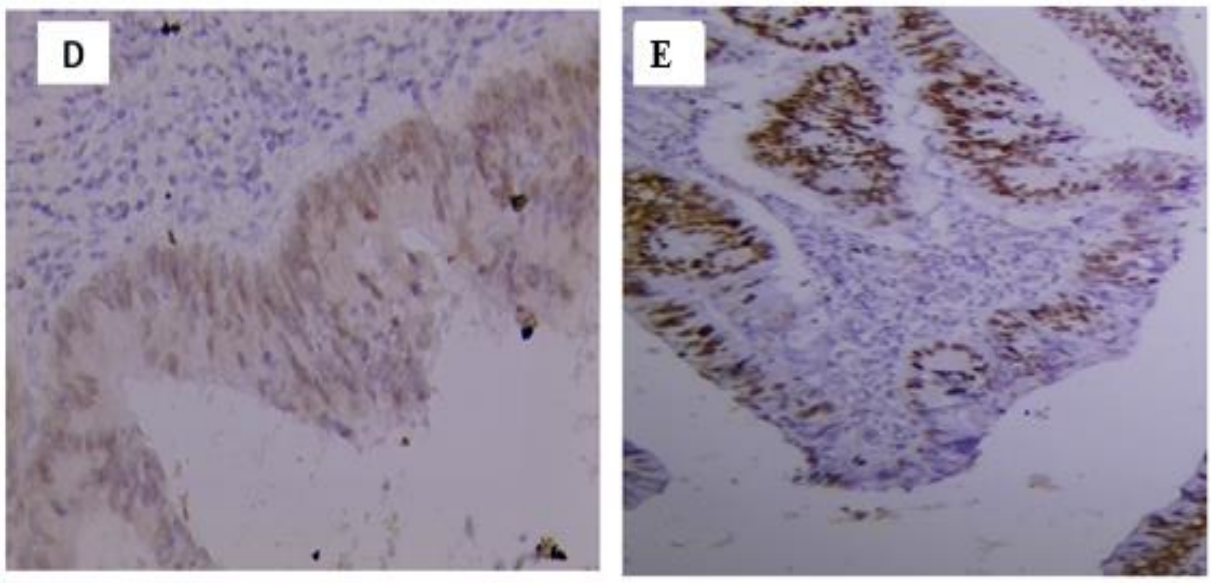

Figure 5: moderate CDX2 expression in colorectal cancer (D), moderate SATB2 expression in colorectal cancer (E) (X40).

Table 3: Correlation between *CDX2* and SATB2 by groups.

Site		Type	Value	*P*p value
Tissue	Tumor	Kendall's tau	+0.667	0.014*
	Colitis	Kendall's tau	0.180	0.172
	Control	Kendall's tau	0.148	0.658

*Significant difference ($P \leq 0.0$)

A high and positive correlation between *CDX2* and SATB2 expression in tumor tissues indicates a significant difference ($p \leq 0.05$) between the two markers. As demonstrated in Table 3, Kendall's tau analysis measured two variables strength and direction of correlation. The correlation coefficient ranged from +1 to -1 in strength, and a (+) sign indicates a positive and complete correlation measures *CDX2* and SATB2 strength and direction in tumor tissues. The expression of SATB2 in 1039 CRCs was compared to that of *CDX2*, with SATB2 showing a higher prognostic power but being lost at a much higher frequency, generally making SATB2 the less sensitive marker for colorectal differentiation [31].

In keeping with findings throughout present study, Elnady *et al*. [12] investigated the validity of SATB2 in the identification of CRCs either alone or in combination with CK20 and/or *CDX2* and showed that combining SATB2 and *CDX2* provided the maximum sensitivity and specificity for detecting CRC. According to Zhang *et al.* [36], from an immunohistochemical perspective, 96 out of 101 (96.0%) and 85.4% for *CDX2* primary CRC samples were SATB2 and *CDX2* positive respectively. IHC-measured SATB2 may also be useful as a diagnostic biomarker for CRC metastases. When SATB2 and *CDX2* were evaluated together as part of a two-marker panel, the detection of metastatic CRCs in liver biopsy tissues was found to have been significantly enhanced. All the previous finding were consistent with the results of the current study.

Neri *et al*. [37] showed that there was no statistically significant connection between SATB2 and any other individual phenotypic marker *(CDX2*), despite the fact that the vast

majority of SATB2-positive cases in CRC were also *CDX2*-positive which disagrees with the results of present study. In the opinion of Dabir *et al.* [38], *CDX2* is a sensitive marker compared to SATB2. Sensitivity for *CDX2* in metastasis from colorectal adenocarcinomas was found to be 93%; while that for SATB2 it was 79%. The specificity of combination of *CDX2* and SATB2 was high for metastasis from colorectal adenocarcinoma. SATB2 can be used as a supplementary marker along with *CDX2* to identify colorectal origin for material received from patients clinically presenting with metastasis all findings are consistent with the current study.

Conclusion

The results showed that *CDX2* and SATB2 expression rates were high in primary CRCs and that there were substantial differences between the various criteria used for assessment. Expression of both SATB2 and *CDX2* was found to be useful as a diagnostic marker for CRC for the prognosis of patients with CRCs. A positive correlation between the two studied markers was also detected. A statistical evaluation of the diagnostic utility of the combined markers was not performed, which could be assessed using a combined ROC curve test.

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