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LEVEL OF CIRCULATING CELL-FREE DNA IN THE SERUM OF EGYPTIAN PATIENTS AS A BIOMARKER FOR DIAGNOSIS AND PROGNOSTIC PREDICTION OF COLORECTAL CANCER

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ABSTRACT

Background: There is a small amount of circulating DNA in the plasma of healthy individuals. Elevated circulating levels of DNA are related to many diseases and their characteristics such as cancer, pregnancy, autoimmune disease, myocardial infarction, trauma and so on. **Aim of the study:** To evaluate the level of circulating cell-free DNA as a serum biomarkers in diagnosis and progression of colorectal cancer (CRC) operated and non-operated Egyptian patients. **Patient and Methods** A case control study was carried out from the periods at July 2015 to December 2016 The study included 90 persons who fulfilling the designed inclusion criteria were classified into four groups, Group I included 30 patients with colorectal cancer (pre-operative), Group II included 30 patients with colorectal cancer (post-operative), Group III included 15 patients with colorectal polyps, and Group IV included 15 healthy volunteers as control group, All participant after detailed history and clinical examination were subjected to routine laboratory investigation, imaging study, endoscopic assessment and serum evaluation of the serum level of circulating cell-free DNA and Carcin-Embryonic Antigen (CEA). **Results**: The mean DNA concentration was 2.46 µg/ml, 0.519 µg/ml 1.684 µg/ml and 1.068 µg/ml for CRC (pre-operative), CRC (post-operative), colorectal polyps and healthy controls participant respectively. DNA concentration was significantly higher in CRC (pre-operative) patients compared with healthy controls (P =0.05). The sensitivity, specificity, positive and negative predictive values of DNA concentration were 36.7% and 100%, 100% and 50.5% respectively which was of value in distinguishing CRC patients from healthy controls. As regard to progression of DNA level the serum concentration in G II was significantly decreased (0.519 ng/dl) versus its level in GI (2.46 ng/dl) p =0.01. Regarding to level of CEA, the results showed that there was decrease in its level in G II (60.03 ng/dl) versus its level in GI g 84.95 ng/dl (p=0.001). **Conclusion**: Level of circulating cell-free DNA could be clinically used as a tool for diagnosis, and follow-up of CRC patients and in discriminating such patients from healthy subjects or patients with benign colonic lesions.

KEYWORDS: Circulating cell free DNA , CRC.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Colorectal cancer represents about 12% of all cancers **(National cancer statistics 2005).** It is the third most common cancer in women after breast and lung cancer whereas in men it also ranks third after prostate and lung cancer, in Egypt, the incidence of colorectal cancer ranges between 2 and 6 percent of the total number of cancer cases reported annually **(Abd al salam, 2010).** Our aim of the study To evaluate the level of circulating cell-free DNA as a serum biomarkers in diagnosis and progression of colorectal cancer (CRC) operated and non-operated Egyptian patients.

PATIENT AND METHODS

A case control study was carried out from the periods between July 2015 to December 2016 to evaluate the level of circulating cell-free DNA as a serum biomarkers in diagnosis colorectal cancer (operated and non-operated patients) and comparing its levels with those obtained from patients with benign lesion as colorectal polyps. The study included 90 persons who fulfilling the designed inclusion criteria they were selected from outpatient clinic of Tropical medicine, Internal Medicine, oncology and oncosurgery departments faculty of medicine, Al-Azhar university hospitals. The patients were classified into four groups, Group I included 30 patients with colorectal cancer (pre-operative), Group II included 30 patients with colorectal cancer (post-operative), Group III included 15 patients with colorectal polyps, and Group IV included 15 healthy volunteers as

control group, All participant after detailed history and clinical examination were subjected to routine laboratory investigation, imaging study, endoscopic assessment and serum evaluation of circulating cell-free DNA and Carcin-Embryonic Antigen (CEA). Data was analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values, and p-value < 0.05 was considered significant.

RESULTS

Table (1): Pattern of serum CEA level among the studied groups.

Group	N	Median (25^{th} -75^{th})	P. Value		
			[a]P	[b]P	[c]P
Group I	30	84.95 (55.15 - 409.8)	0.001**	0.001**	
Group II	30	60.03 (53.33 - 75.8)	0.001**	0.001**	0.001**
Group III	15	15.15 (10.7 - 54.27)	0.3		
Group IV	15	31.16 (14.79 - 41.66)			

[a]p= Another groups compared with Group IV. ** is a highly significant at the p<0.001
[b]p= Group I and Group II compared with Group III. * is significant at the p<0.05
[c]p= Group II compared with Group I.
P value was considered significant at <0.05
Data are represented by median (25^{th}-75^{th} percentile)

As regard pattern of serum CEA level among the studied groups this table showed that the median level of CEA was significantly higher in in group I patients (84.95ng/ml) when compared to that of the group II (60.03 ng/ml) (p=0.001), group III (15.15 ng/ml) (p=0.001) and group IV (31.16ng/ml) (p=0.001).

Table (2): Pattern of serum DNA level among the studied groups.

Descriptive Statistics of Serum DNA								
Group	N	Min	Max	Mean	S.D	P. Value		
						[a]P	[b]P	[c]P
Group I	30	0.1	14.7	2.46	2.1	0.05*	0.3	
Group II	30	0.001	2.16	0.519	0.52	0.002**	0.001**	0.01*
Group III	15	0.32	2.7	1.684	0.87	0.02*		
Group IV	15	0.29	1.62	1.068	0.53			

[a]p= Another groups compared with Group IV. ** is a highly significant at the p<0.001
[b]p= Group I and Group II compared with Group III. * is significant at the p<0.05
[c]p= Group II compared with Group I.

As regard pattern of serum DNA level among the studied groups this table showed that the mean level of DNA concentration was statistically higher in group I patients (2.46 ng/ml) when compared to that of the group II (0.519 ng/ml) (p=0.01), group III (1.684 ng/ml) and group IV (1.068ng/ml) (p=0.05).

But there is no statistically significant difference.

Table (3): Correlation between DNA in relation to prognostic factors among the CRC groups

Serum DNA according the following parameters		CRC Median (25^{th} -75^{th})	P. value
Grading of the tumor	Stage II	0.62 (0.26 - 3.5)	0.04*
	Stage III	0.48 (0.24 - 2.56)	
Histopathologic type of CRC	Adenocarcinoma	0.53 (0.24 - 2.56)	0.01*
	Mucinous adenocarcinoma	0.86 (0.26 - 4.5)	
Site of CRC	Transverse colon	4.8 (0.45 - 13.29)	0.5
	Distal colon (Rectum + Rectosegmoid)	0.87 (0.25 - 2.98)	
	Proximal colon(Caecum)	0.33 (0.19 - 0.62)	
	Ascending colon (Hepatic flexure)	0.19 (0.14 – 0.28)	
Lymph nodes	No	0.62 (0.26 - 3.46)	0.9
	Yes	0.48 (0.24 - 2.56)	
Status of distant metastasis	Evidence	0.53 (0.24 - 2.98)	0.01*
	No evidence	0.33 (0.2 - 0.97)	

P value was considered significant at <0.05
Data are represented by median (25^{th}-75^{th} percentile)

As regard correlation between DNA in relation to prognostic factors among the CRC groups this table showed that there was statistically significant difference was found between median level of DNA and the grading of the tumor (p=0.04), histopathological type of the tumor (p=0.01) and distant metastasis of the tumor (p=0.01).

But there was no statistically significant difference was found between median level of DNA and site of the tumor (p=0.5) and L. Ns metastasis (p=0.9).

Table (4): The Diagnostic Significance for studied Markers to Diagnose the CRC among the studied groups.

		Markers cutoff	AUC	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)	Diagnostic accuracy
CEA	Polyp with control	41.8	45.3	40.0	86.7	75.0	59.1	63.3
	Preoperative with polyp	54.7	86.0	76.7	86.7	92.0	65.0	80.0
	(CRC) Preoperative with control	54.4	94.9	76.7	100.0	100.0	68.2	84.4
Serum DNA	Polyp with control	1.62	79.0	60.0	100.0	100.0	71.4	80.0
	Preoperative with polyp	2.7	35.3	26.7	100.0	100.0	40.5	58.7
	(CRC) Preoperative with control	1.62	40.0	36.7	100.0	100.0	40.5	60.0
Serum DNA and CEA	CRC with Control			85.0	25.0	64.0	20.0	80.0

As regard the Diagnostic significance for studied markers to diagnose the CRC among the studied groups this table showed that combination of Absolute DNA concentration and CEA raised the sensitivity to reach 85% and diagnostic accuracy to reach 80% which was better than either of them alone.

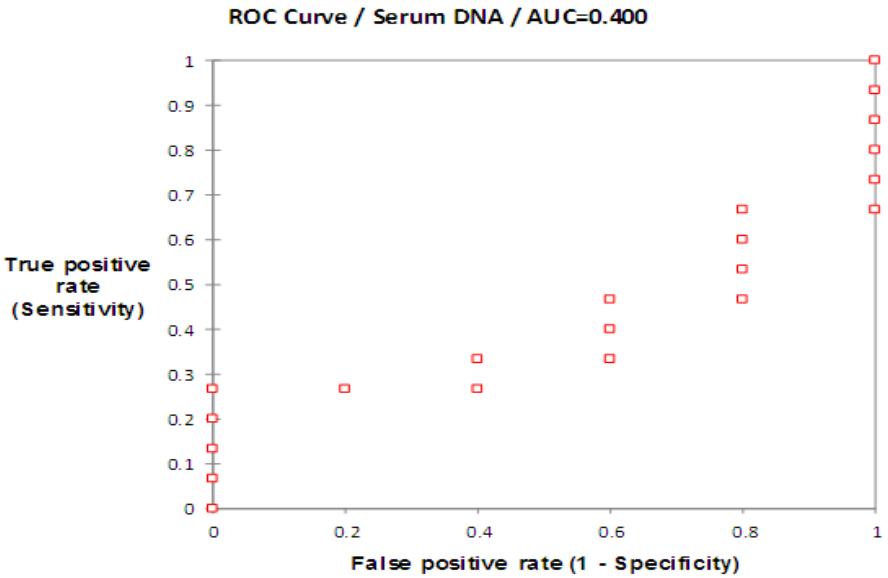

Figure (1): ROC curve analysis was done to estimate the diagnostic value of serum DNA level in discriminating patients with Pre-Operative group from control group.

Table (5): Diagnostic value of serum DNA level in discriminating patients with Pre-operative group from control group.

Serum DNA	Sensitivity	Specificity	PPV	NPV	TP	TN	FP	FN	Accuracy
1.62	36.7	100.0	100.0	50.5	8	15	0	22	60

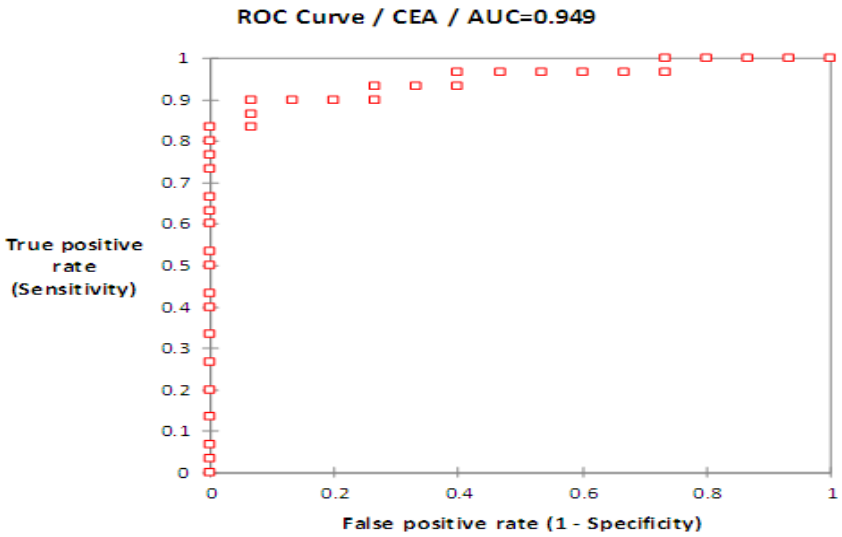

Figure (2): ROC curve analysis was done to estimate the diagnostic value of serum CEA level in discriminating patients with CRC (pre-operative) group from control group.

Table (6): Diagnostic value of serum CEA level in discriminating patients with pre-operative group from control group.

CEA	Sensitivity	Specificity	PPV	NPV	TP	TN	FP	FN	Accuracy
54.4	76.7	100.0	100.0	68.2	23	15	0	7	84.4

DISCUSSION

Colorectal cancer (CRC) is a major health problem. Worldwide, more than one million individuals develop CRC each year **(Ferlay et al., 2013).** It is one of the few preventable cancers and cancer related survival is closely related to the clinical and pathological stage of the disease at time of diagnosis **(Otero et al., 2015).** Our aim in the current cross sectional study was to evaluate the level of circulating cell-free DNA as a serum biomarkers in diagnosis and progression of operated and non-operated Egyptian patients with colorectal cancer (CRC). The mean age among the studied patients was 46, 44 and 46 in GI, GII, and GIII respectively the results agree with that is reported by **Gado et al 2014** they reported that the mean age of patients was 51 years with 25% of cancers occurring in patients aged less than 40 years. Our data are similar to those reported in other Middle-Eastern countries and are much higher than in Western countries. Reports from Middle-Eastern countries show a higher prevalence of CRC in patients undergoing colonoscopy than in the West. CRC was detected in 2.1% of patients who underwent colonoscopy in the United Kingdom and 9–11% in Morocco and Sudan. **(Thomas et al., 2002) (Hassan et al., 2008) (Mudawi et al., 2010).** Also reports from Middle-Eastern and African countries show higher CRC rates in younger patients than in the West. CRC was diagnosed in patients aged 40 years or younger in 2–6% of CRC cases in Italy, France and Taiwan and in 17–36% in Saudi Arabia, Sudan and Iran. **(Pahlavan and Kanthan., 2006) (Cascinu et al., 1996) (Lee et al., 1994).** In Egypt reports showed that CRC was detected in 11–15% of patients who underwent colonoscopy and diagnosed in 29–31% of patients aged 40 years or younger. **(Zakaria et al., 2006) (Khafagy et al., 2000).**

The male gender in CRC group was 20 patients 66% and 10 patients 33% in GI and GII respectively while in benign group was all participants were male. The results were in agreement with that is reported by **Richardson, (2011)** they reported that male incidence rates in CRC group were higher than that for females. Also, the latest colorectal cancer report from American Cancer Society demonstrated that the lifetime probability of a colorectal cancer diagnosis is 4.7% in women and 5.0% in men. Also, these results were in agreement with **(Siegel et al., 2014)** they reported that incidence and mortality rates were 30% to 40% higher in men than in women overall and the male to female incidence rate ratio (IRR) varies by age. The results were in agreement with retrospective study from Thailand that demonstrated both benign colorectal tumors and CRC were more commonly found in males (63%) than females (37%) **(Kotepui et al., 2013).** The reason for higher rates in men is not entirely clear, a potential reason is that men have a higher exposure to risk factors, including physical inactivity, excess body weight, high intake of red meat, high alcohol intake, and cigarette smoking **(Giovannucci, 2002; Larsson and Wolk, 2006).** In addition, exposure to endogenous or exogenous estrogen may also contribute to a lower incidence in women. Observational studies have demonstrated a reduced risk of colorectal cancer among women taking postmenopausal hormones **(Grodstein et al., 1999).** But, these results disagree with those reported by **Kanate et al., (2013)** who showed in a clinical study that women in developed countries have the same risk of CRC as men. Also, **Umetani et al., (2006)** and **Bhandari et al., (2011)** stated that there was no statistically significant difference regarding age or sex

groups. Also, **Storm et al., (2013)** showed in a clinical study that men have the same risk of CRC as women.

The main clinical presentation among the studied patients with CRC was bleeding per rectum in 18 patients 60%) and 7 patients (24%) presented with manifestation of anemia, but change of the bowel habits were 3 patients (10%). the results is in agreement with Egyptian study by **Hoda 2010** she reported that the Bleeding per rectum and abdominal pain were the most common symptoms constituting 54% and 42.7% of all patients respectively. Also, our results were in agreement with **John et al., (2013)** who reported that the commonest symptoms were rectal bleeding (25.5–42.3%) and change in bowel habit (20.6–26.8%) while low risk symptoms were abdominal pain (16.3–46.8%) and weight loss (18.4–26.1%). Also, these results were in agreement with **Storm et al., (2013)** who reported that a significant proportion of CRC patients present with normocytic anemia (18%).

Regarding to site of the tumor among patients of G I (CRC group) in the current study reveals that 18 patients (60%) in distal colon (sigmoid colon & rectum), 6 patients (20%) at proximal colon (caecum), 4 patients (13%) at the transverse colon, 2 patients (7%) at the ascending colon. These results were in agreement with **Dina et al., (2016)** who found that site of the tumor was 50% in the distal colon (sigmoid colon & rectum) and 50% in the proximal colon and distal colon (Cecum, ascending colon, transverse colon, descending colon). The rectum is the most common site to be involved where 60% of all patients had rectal tumors. In the early Egyptian publication from NCI, rectal carcinoma constituted 75-79% of cases **(Kenawi et ai., 1999)** In the later Egyptian series, the rectum constituted 68% of all CRC **(Khafagy et al., 2000).** Conversely, the result of this study is different from other Egyptian publication where the distal colon was the most common site contributing 50.2% of cases followed by the rectum which constituted 27% of cases **(EL-Bolkainy et al., 2005).** Studies from different regions have documented almost similar figures where more than 50% of malignancy is present in the rectosigmoid **(Kan et al., 2004) (AL-Jaberi et al., 2003).** However, Our results disagreed with American colorectal cancer statistics report which showed that the most common tumor location was the proximal colon (42%), followed by the rectum (28%). Also **Siegel et al., (2014)** is not matched with our results, he stated that percentage of the tumor were higher 46% at the proximal colon and 24% at the rectum.

As the main aim of our study to evaluate the level of circulating cell-free DNA as a serum biomarkers in diagnosis and progression colorectal cancer. The current study showed that the median level of DNA concentration were significantly higher in CRC group **(2.46 ng/ml)** when compared to its median level in control group **(1.068 ng/ml) (p<0.05).** The finding demonstrates that the plasma DNA level in patients with cancer is significantly higher than that in the normal individual. The finding may due to several explanations (I) cells actively secreting or releasing DNA during DNA replication, whereby active proliferation of cancer cells result in the sustained release of newly synthesized DNA fragments into the extracellular and entering the blood circulation; (II) the apoptosis of tumor cells: apoptosis is characterized by DNA degradation. **(Ellinger et al., 2008)** (III) the inhibition of the enzymes responsible for degradation of DNA: the small amount of free DNA in normal human plasma is rapidly degraded, likely by DNAse I or II; but in the malignant tumor, this enzyme activity may be inhibited (e.g., by a very strong inhibitor of DNAseI or II in blood circulation). It results in the buildup of the level of DNA in plasma in patients with malignant tumors **(Silva et al., 1999);** (IV) circulating tumor cells in blood: some scholars put forward that the free circulating DNA is released into the bloodstream by intact tumor cells and the lysed tumor cells **(Schwarzenbach et al., 2009).** These results were in agreement with **Umetani et al. (2006)**, **Mead et al., 2011, Leszinski et al. 2013** and **Zaher et al., 2014** they found statistically significant difference between CRC group and the healthy control subjects **(p<0.01).** Also **Maio in 2014** reported that free circulating DNA is considered to be a derivative of increased and abnormal apoptotic pathways in the cancerous lesions. The abnormal DNA degradation leads to increased DNA levels and DNA fragments of different sizes **(Leszinski et al., 2013).** Several studies use plasma to quantify the circulating cell-free DNA, while other studies use serum as a template. Moreover, some studies performed DNA extraction **(Agostini et al., 2011)** and measured the levels of circulating cell-free DNA by qPCR, while other studies use serum **(Umetani et al., 2006)** or plasma **(Mead et al., 2011)** as a direct template to quantify cell-free DNA. Moreover, patients in the current study with distant metastasis at the time of presentation showed statistically significantly higher median level of cf DNA **(p = 0.01)** in GI versus non-significant difference in the level of CEA in the same group **(p = 0.1)** The biological characterization of circulating DNA in the blood of patients has shown that an important component derives from tumor cells **(Garcia-Olmo., 2001) (Johnson and Lodym., 2002).** Also, patients in the current study with distant metastasis at the time of presentation showed significantly higher median level of DNA concentration than that in non-metastatic patients at **(p=0.01).** These results were in agreement with **Dina et al., (2016)**, who found statistically significant difference between the DNA concentration with distant metastasis than that in non-metastatic patients **(p=0.004).** However these results disagreed with **Zaher et al., (2014)** who reported that there was no significant association between state of distant metastasis and DNA concentration in CRC patients. Median level of CEA in the current study was 84.95, and 60.03 in GI and GII versus 15.15 and 31.16 in GIII and G IV respectively. As CEA, the most widely used serum marker in colorectal cancer, offers a

sensitivity of only 30% to 40% for early-stage tumors **(Fernandes et al., 2005)**, also confirmed in our study. A large number of studies have reported higher concentrations of serum/plasma free DNA in patients with various types of cancer **(Sozzi et al., 2001) (Gautschi et al., 2004) (Tong and Lo., 2006).**

The diagnostic accuracy of CEA and free DNA was evaluated using continuous values in ROC curve analysis. The area under the ROC curve (AUC) was **0.949** for CEA and **0.004** for free DNA. Sensitivity and specificity were calculated for different cutoff values using the standard 5 ng/mL for CEA and various cutoff values for free DNA. Sensitivity and specificity for **CEA** were **76.7%** and **100%** respectively. The results also agree with that is reported by **Emanuela et al., 2006** who reported that the AUC is 0.82 for CEA and 0.86 for free DNA. Sensitivity and specificity for CEA were 38.7% and 97.3% respectively. The serum marker CEA is currently the tumor marker in use for colon cancer diagnosis and follow-up. Median level of CEA was significantly higher in CRC group **(84.95 ng/ml)** when compared to both benign group **(15.15 ng/ml)** and control group **(31.16 ng/ml) (P=0.001) (p=0.001).** These results were in agreement with **Dina et al., (2016)** who reported that level of CEA concentrations were significantly higher in CRC group than that in the benign and the control groups. **Rong et al.(2014)** who studied 64 patients with CRC and 36 subjects with non-malignant colorectal disease, founded that significant difference between both groups.

In the current study, there was significant positive correlation between DNA concentration and tumor marker CEA **(P<0.001) (r=0.867).** Our results were supported by following studies: CEA is the most specific polysaccharide‑protein complex which contributes to the malignant characteristics of the tumor **(Goldstein and Mitchell., 2005).** It is not usually present in the blood of healthy adults although levels are raised in heavy smokers. It is also a potential marker for monitoring response to chemotherapy. Several studies have shown that patients who exhibited a decrease in CEA whereas on chemotherapy had a better overall survival compared with those whose CEA concentrations failed to decrease **(Duffy., 2001).** Also, these results were supported by **Umetani et al. (2006).**

On comparing the DNA concentration with tumor grade; Grades (III and IV) showed statistically higher DNA concentration than that of grade II **(p=0.04).** The results were supported by **(Ellinger et al., 2008),** he reported that cells actively secreting or releasing DNA during DNA replication, whereby active proliferation of cancer cells result in the sustained release of synthesized DNA fragments into the extracellular and entering the blood circulation; However these results disagreed with **Umetani et al., (2006)** and **Zaher et al. (2014)** they found that there was no significant difference between the DNA concentration and tumor grading, having the same median level. No statistically significant difference was found in the median level of DNA concentration in different states of lymph node **(p=0.9)** or site of the tumor **(p=0.5)** which were in agreement with **Dina et al., (2016)**, who found no statistically significant difference between the DNA concentration and lymph node metastasis **(p=0.6)** or site of the tumor **(p=0.4).** Also, these results were supported by **Zaher et al., (2014).**

Receiver operating characteristic curves (ROC) comparison demonstrated that DNA levels displayed low sensitivity and high specificity in identifying patients with CRC from healthy controls. In the present study Comparing DNA level in CRC patients with healthy controls, DNA level was found to be low sensitivity of **36.7%** and highly specificity of **100 %** with positive predictive value of **100 %** and negative predictive value of **50.5 %** in differentiating patients with CRC from healthy controls with cutoff value **1.62 ug/ml** and **60%** accuracy. These results were in agreement with **Dina et al., (2016)** who reported that ROC curve for absolute DNA concentration showed AUC = 0. 73 and at cutoff value of 3.3 ng/µl had a sensitivity of 68%, specificity of 65% and diagnostic accuracy of 57.2%. Also, these results were in agreement with that showed by **(Umetani et al., 2006)** where ROC curve for absolute DNA concentration showed AUC =0.75 at 1.73ng/µl showed sensitivity of 40% and specificity of 90%. Also, In the present study Comparing DNA level in CRC patients with benign group, ROC curve for absolute DNA concentration showed that AUC**=0.353**, low sensitivity of **26.7%** and high specificity of **100 %** with positive predictive value of **100 %** and negative predictive value of **40.5 %** in differentiating patients with CRC from healthy controls with cutoff value 2.7 ug/ml and 58.7% accuracy. These results were slightly similar to that showed by **Dina et al.,(2016)** where ROC curve for absolute DNA concentration showed AUC = 0.83 and at cutoff value of 2.35 ng/µl had a sensitivity of 84%, specificity of 70% and diagnostic accuracy of 81.7%. Also, these results were similar to **(Mead et al., 2011).**

In the present study Comparing CEA level in CRC patients with healthy controls, ROC curve for CEA showed that AUC**=0.949** and at cut off **54.4 ng/ml** with sensitivity of **76.7%,** specificity of **100%,** positive predictive value of **100 %,** negative predictive value of **68.2 %** and diagnostic accuracy of **84.4%**. These results were slightly similar to **Dina et al., (2016)** where ROC curve for absolute CEA yield an AUC of 0.86, at cutoff 2.1 ng/ml, sensitivity of 82%, specificity of 80% and diagnostic accuracy of 81.5%. Also, these results were slightly similar to **Rong et al., (2014)** who showed that ROC curve for CEA had AUC: 0.88, at cutoff 3.21ng/ml had sensitivity of 80.43% and specificity of 75.00%. Also, In the present study Comparing CEA level in CRC patients with benign group, ROC curve for CEA concentration showed that AUC **= 0.860,** at cutoff **54.7 ng/ml,** sensitivity of **76.7%,** specificity of **86.7%** positive predictive value of **92 %,** negative predictive

value of **65 %** and diagnostic accuracy was **80%.** These results were slightly similar to **Dina et al., (2016)** where ROC curve for absolute DNA concentration yield an AUC of 0.89, at cutoff 2.0 ng/µl, it had a sensitivity of 84%, specificity of 90% and diagnostic accuracy of 85%. Also, these results come in agreement with **(Mead et al., 2011).** Also, In the present study Comparing CEA level benign group with healthy controls, ROC curve for CEA concentration showed that AUC**=0.453** at cut off **41.8 ng/ml,** sensitivity of **40 %** specificity of **86.7%,** positive predictive value of **75%,** negative predictive value of **59.1%** and diagnostic accuracy of **63.3%.** These results were slightly similar to **Dina et al., (2016)** where ROC curve for CEA yield an AUC of 0.78, at cutoff 0.28 ng/ml, it had a sensitivity of 54%, specificity of 80% and diagnostic accuracy of 65%.

Combination of currently used marker CEA with absolute DNA concentration had positive impact on the diagnostic value. Currently used marker CEA was shown in current study to have lower diagnostic value for CRC than both absolute DNA concentrations in agreement to that previously reported by **Mead et al., 2011**. Combination of Absolute DNA concentration and CEA raised the sensitivity to reach 85 %and diagnostic accuracy to reach 80% which was better than either of them alone. These results were slightly similar to **Dina et al., (2016)** which report that combination of absolute DNA concentration and CEA increased the sensitivity to reach 100% and diagnostic accuracy to reach 93.3% better than either of the two markers alone. Also, these results were close to Mead et al.2011 who found that the combined DNA marker and CEA showed AUC of 0.85, sensitivity of 83%, specificity of 72% better than either alone.

CONCLUSION

We concluded that DNA concentration could be clinically used as a tool for diagnosis, and follow-up of CRC patients and in discriminating such patients from healthy subjects or patients with benign colonic lesions.

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