Original Article

The value of serological markers (CA19-9 & CCSA4) in the screening and prognosis of colon cancer

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Abstract:

Background:- Colonic cancer is a very common disease world-wide being fourth most common cancer characterized by abnormal proliferation of the inner wall of colon at beginning then taking full colon wall thickness then to surrounding lymph nodes and tissues and finally metastasis.

Objective:- To evaluate the efficacy of serum CA19-9 and CCSA-4 levels in the screening and prognosis of colonic cancer and their validity for this.

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Patients and methods:-This study was applied on 35 patients with colonic cancer, 35 patients with benign polyps and 16 negative controls. All individuals were subjected to blood sampling for measuring their serum CA19-9 and CCSA-4 using ELISA technique.

Results:-Our study shows that colonic cancer patients presented at ages between 53-82 years of age (mean 68.5). Serum levels of CCSA-4 were significantly elevated in those patients with advancing stages (C&D) compared with stages (A&B) and lower levels found in patients undergone surgical removal of tumor or received chemotherapy.Also a positive relation found between CCSA-4 with alcohol intake and smoking. Less significant levels and relations found with CA19-9.

Conclusion:-Serum CCSA-4 is sensitive and specific indicator for diagnosis, prognosis and screening of colonic cancer.

Keywords:-Colonic cancer, tumor marker, CA19-9, CCSA-4.

Introduction:-

Colon cancer is large intestinal cancer characterized by vague symptoms at the beginning such as :changes in bowel habits, including diarrhea or constipation ,change in the consistency of the stool ,rectal bleeding or malena ,persistent abdominal discomfort ,such as cramps ,gas or pain ,tenesmus ,fatigue and unexplained weight loss.[1] However ,colon cancer usually begins as small benign (non-cancerous) polyps that may produce little ,if any ,symptoms ,thus doctors when discover polyps should screen patient periodically ,as it may convert into cancer.[2] Screening can be done by measuring serum levels of certain tumor markers including :CA19-9 ,CEA, CCSA-3 & CCSA-4.[3] Diagnosis of colon cancer is based on endoscopic examination and biopsy from suspected areas to be then sent for histopathological examination or by biopsy obtained after surgical removal of affected part of colon in addition to signs and symptoms ,if any .Still stool Deoxy-ribo Nucleic Acid (DNA) test plays an important role in early diagnosis ,while screening and prognosis are determined by follow-up the serological levels of tumor markers such as CEA, CA19-9 ,CCSA-3and CCSA-4[4,5]. However ,still these tumor markers also raise in other non-cancerous conditions such as:Inflammatory bowel disease ,polyposis and others. Thus studies are conducted to investigate the value of using cluster of differentiation (CD) markers in screening and prognosis of colon cancer [6,7].Colon cancer has affected civilizations since centuries.At 2007 an article published in medical journal including how ancient Chinese used certain herbal remedies to treat colon cancer about 6000 years ago (Ballard-Barbash R. , 2007). On the other hand, ancient Greeks used olive oil as preventive measure while traditional Indians have used mustard to relieve colonic symptoms [8].In 1913 an American pathologist Aldred Scott Warthin whom discovered hereditary link to some types of colon cancer which is now known as Lynch syndroms 1&2 [9].An English pathologist Cuthbert Dukes have created at 1932 the staging of colon cancer which is still used until now [10].So, it is hard to say that there is a single person who is responsible for colon cancer discovery, rather it is a group effort throughout the world that with the advances in medical researches and equipment have reached the recent definition of symptoms, signs and causes of colon cancer [11].Colon cancer is the fourth most common cancer and the second most common cause of death worldwide preceded only by heart disease [12,13].For Iraq colon cancer is the 7th. Most common cancer affecting males more than females, especially smoker males [14].Highest incidence rates countries include:

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Australia, New Zealand, Canada, the United States, and parts of Europe , while the countries with the lowest risk include: China, India, and parts of Africa and South America [15].In Iraq the incidence of colon cancer is increasing because of the change in dietary habits, environmental factors and the hazard of the chemicals of wars that are still seen until now [16].The geographic differences appeared attributable to the difference in dietary and environmental exposures that are imposed upon a background of genetically determined susceptibility. On the other hand, low socioeconomic status (SES) also appeared to be associated with an increased risk for the development of colonic cancer [17,18].

Potentially modifiable behaviors such as physical inactivity, unhealthy diet, smoking, and obesity are thought to account for a substantial proportion (estimates of one-third to one-half) of the socioeconomic disparity in risk of new onset colonic cancer [19].In general colonic cancer incidence didn't show striking differences between different races. But different studies have showed higher incidence within same race over years, while survival rates were higher in Caucasians over Africo-Americans [20,21,22].

Materials and Methods:-

A study conducted on the following groups in the period between October 2015 and June 2016. 35 male and female colonic cancer patients attended " Baghdad medical city Teaching Hospital" and "Gastroenterology and Liver Diseases center in Baghdad Teaching Hospital" , 35 male and female patients with benign colonic adeno-polyposis and 16 apparently healthy people with negative findings to any benign or malignant colonic disease of any type.Blood samples were taken from each individual by venous puncture, left to clot then centrifuged and serum collected in (2) aliquots and stored at -20 C, then detect CA19-9 & CCSA-4 by ELISA technique. CA19-9 or CCSA-4 ELISA kits: This assay employs an antibody specific for human CA19-9 or CCSA-4coated on a 96 well plate. Standards and samples are pipetted into the wells and CA19-9 or CCSA-4 present in a sample is bound to the wells by the immobilized antibody.The wells are washed and biotinylated antibody, horseradishperoxidase (HRP) conjugated streptavidin is pipetted to the wells. The wells are again washed. Tetramethyl-benzidine (TMB) substrate solution is added to the wells and color develops in proportion to the amount of CA19-9 or CCSA-4 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm by spectrophotometer. The amount of color is directly proportional to the concentration of CA19-9 or CCSA-4 antibody present in the original sample.

Statistical analysis:-Dataexpressed as mean ±SD. Statistical differences between the groups were determined according to ANOVA test and student t-test was considered to be significant <0.05.

Results:-

Table 1: The difference in mean age between the 3 study groups.

Age (years)	Study group		
	Negative control	Benign polyp	Cases (Colonic Cancer)
Range	(25 to 43)	(29 to 56)	(53 to 82)
Mean	32.9	41.9	68.5
SD	5.0	7.9	6.4
SE	1.2	1.4	1.1
N	16	32	35

*P (ANOVA) <0.001

Table 2: The median serum CA19-9 and CCSA-4 by chemotherapy.

	Chemotherapy		
	Negative	Positive	P
CA19-9			0.23[NS]
Range	(11 to 376.6)	(11.3 to 42)	
Median	18	39.1	
Interquartile range	(13.9 to 20.9)	(14.9 to 40.9)	
N	29	6	
Mean Rank=	17.1	22.6	
CCSA-4			0.009
Range	(11.5 to 154.6)	(13.4 to 19.4)	
Median	29.4	15.25	
Interquartile range	(17.5 to 51.6)	(13.6 to 17.5)	
N	29	6	
Mean Rank=	20.1	8.1	

Table 3: The median serum CA19-9 and CCSA-4 by surgical removal of the primary tumor.

	Surgical removal of the tumour		
	Negative	Positive	P
CA19-9			
Range	(11 to 293.8)	(11 to 376.6)	
Median	18.05	20.9	
Interquartile range	(13.9 to 31.2)	(14.9 to 39.4)	0.64[NS]
N	30	5	
Mean Rank=	17.7	20	
CCSA-4			
Range	(13.4 to 154.6)	(11.5 to 20.2)	
Median	28.75	14.6	
Interquartile range	(17.5 to 51.6)	(13 to 16.2)	0.007
N	30	5	
Mean Rank=	19.9	6.6	

Table 4: The median serum CA19-9 and CCSA-4 by alcohol consumption habit.

	Alcohol		
	Negative	Positive	P
CA19-9			
Range	(11 to 376.6)	(13.9 to 32)	
Median	18.1	23.95	
Interquartile range	(13.9 to 38.4)	(15.3 to 31.6)	0.82[NS]
N	31	4	
Mean Rank=	17.9	19.1	
CCSA-4			
Range	(11.5 to 154.6)	(21.2 to 149.2)	
Median	19.4	53.5	
Interquartile range	(16.2 to 36.8)	(36.4 to 102.3)	0.043
N	31	4	
Mean Rank=	16.7	27.8	

Discussion:-

The current study showed that the mostly affected group with colonic cancer are those aged between (53-82) years of age) average 68.5 years ,(with higher values of serological markers) especially CCSA (4-in older patients compared to younger ages with) P , (0.001 > this result agrees with many other studies) Ossein (2014,which considered that the average age of diagnosis for colonic cancer is72 years of age90% , of new cases and 95% of deaths from colon cancer occur in people 50 years age or older .Also) Rick Alteri and PritiBandi, (2015held a study in America concluded that colon cancer incidence is 15 times higher in ages 50 years and older compared to younger ages . In a previous study in Iraq at ,2015the peak age of incidence of colon cancer was(60-69) years age group ,A study held in United Kingdom) U.K (.at ,2014considered that most colonic cancers occur at ages70 and older .Also a study held on American citizens showed that 70%of the deaths from colonic cancer patients are aged65 years of age and older.[23,24,25,26,27]Still ,other study held in Italy at 2015 showed that incidence of colonic cancer is declining in people about 50 years and older ,while increasing rates occur in younger ages.[28] The current study found significantly higher CCSA 4-levels in those with colonic cancer which is more sensitive and specific than CA19-9, this is because CA19-9 also increases in other malignancies as pancreatic ,hepatocellular , ovarian and other cancers in addition to colorectal cancer, also it increases in nonmalignant conditions such as :pancreatitis ,cirrhosis ,cholangitis ,ovarian cyst , poorly controlled diabetes mellitus and many other benign conditions thus it is considered of low sensitivity and specificity .A study held on europian population showed that there is 42% patients with colonic cancer has elevated CA19-9 levels ,yet other studies concluded that CA19-9 is not even acceptable as prognostic tool for colonic cancer.[29,30] While CCSA 4-increases only in response to colorectal cancer. This agrees with a study held in America shows a sensitivity of) 100% lower 95% confidence bound ,(The sensitivity for detection of the combined end point of colorectal cancer and advanced adenoma for CCSA 4-was ,84.8% The specificity in individuals with normal ,hyperplastic polyps ,or non-advanced adenomas was 91.0% for CCSA .[31,32] 4-The current study concluded that CA19-9 and CCSA 4-are useful in diagnosis ,prognosis and screening of colonic cancer .Also that CCSA 4-is much more sensitive and specific for colonic cancer .Despite years of research and hundreds of reports on tumor markers in oncology ,the number of markers that have emerged as clinically useful is pitifully small.[33] A strong positive relationship found between CCSA 4-and colon cancer stage with) P (0.001 > while CA19-9 found to have weak relation with stage with) P .(0.82 > this agrees with other studies which showed that CA19-9 and CCSA 4-shows higher levels with advancing stage .This agrees with other studies held in Europe which showed increasing CCSA 4-levels with advancing stage.[34] CCSA 4-levels found to be higher in people whom didn‘t receive chemotherapy compared to those whom received with (P = 0.009), while CA19-9 showed less significant difference with) P ,(0.23 = this also agrees with a study held in America showing lower CCSA 4-levels in those receiving chemotherapy.[35] It was also found that patients whom undergone surgical removal of colonic malignant tumor showed lower CCSA 4-levels compared to other patients whom didn‘t ,with (P = 0.007), while CA19-9 showed less significant difference with) P ,(0.64 = this agrees with many previous studies.[36] Patients received Immunotherapy showed significantly lower CCSA 4-levels with) P(0.02 = ,while CA19-9 showed less significant difference with) P .(0.26 =Alcoholic patients showed higher levels of CCSA4-than non-alcoholic with) P , (0.043 = while CA19-9 showed less significant difference with) P ,(0.82 =also close results were found in other studies.[37] Smoker patients had higher CCSA 4-levels compared to non-smokers with) P(0.012 = ,while CA19-9 showed less significant difference with) P= ,(0.33such close studies also found.[38] Our study ,concludes the highest specificity and sensitivity of CCSA 4-measures in colonic cancer patients) at cut-off value (11.4 ≤ being97.9% and 100% respectively ,this result agrees with some studies focused mainly on the validity of serum CCSA 4-in detection of colonic cancer and concluded that CCSA4 sensitivity and specificity were 100% and 95% respectively.[39] While cut-off value for CA19-9 was set at 88.6% , 12.4≤ sensitive and

87.5%specific at this value , this agrees with some studies showing CA19-9 sensitivity and specificity for colonic cancer patients are 76% and 90% respectively.[40] Other studies, documented that CA19-9 lack sensitivity and specificity when used for diagnosis screening or prognosis of colonic cancer. Nevertheless ,researchers continue on the development of blood tests for diagnosis ,screening and prognosis of colonic cancer ,even yet there were available for routine use in clinical practice.[41] Analysis of ROC showed highreceiver operating characteristic curve)ROC (area (0.9<) differentiating colonic cancer from benign colon lesions or negative colon lesions with the CCSA 4-was an almost perfect test with a validity higher than that of CA19-9 ,this agrees with other studiesheld for colon cancer patients showing sensitivity ,26%=area under curve) AUC) 0.580= (with standard error) SE ,(0.05= (with a significance level of P.0.1670=Also ROC curve in other study for CCSA 4-showed AUC of 95%) 0.94 CI ,(0.98–0.90 ,using cut point 0.25 µg/ml ,would generate a sensitivity of94% for detection of colorectal cancer and advanced adenoma but lower the specificity to.[42,43] 84%

Conclusions:-

Serum CA19-9 and CCSA-4 were significantly higher in colonic cancer patients than in both benign polyps and negative controls WITH CCSA-4 being more sensitive and specific. Their levels were very useful in screening and prognosis of the disease. The levels of CCSA-4 appear to be significantly affected by different treatment options including surgical removal, chemotherapy and immunotherapy.There was a strong positive relation between CCSA-4 levels and stage of colonic cancer at time of the test.

AuthorContributions-:

AthraaAhmmadM.Yacoob AL-Obaidy / Acquesition of data analysis, interpretation of data and drafting of manuscript.

Hayfaa Salman AL-Hadithi / Study conception, design, interpretation of data and critical revision.

AqeelShakirMahmood / Acquisition of data analysis and critical revision.

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