Serological markers "CEA test & sAPRIL test" in Iraqi patients with colon cancer

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Fac Med Baghdad 2017; Vol.59, No.4
Received: Aug.2017
Accepted: Dec .2017

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 then taking full colon wall thickness then spreading to surrounding lymph nodes and tissues and finally distant metastasis. It is one of most complicated diseases with debilitating symptoms which becomes more sever , prominent and specific with advancing stage with high percent of fatality and relatively short survival if diagnosed late or if left untreated.

Objective: To evaluate the efficacy of serum CEA & sAPRIL levels in the diagnosis and screening of colon cancer and their validity for this.

Patients and methods: This study was applied on 35 patients with colonic cancer, 35 patients with benign polyps and 15 negative controls. All individuals were subjected to blood sampling for measuring their serum CEA & sAPRIL using ELISA technique.

Results: In this study, majority of patients with colon cancer were presented at ages between 53-82 years of age (mean 68.5±6.4 years). Serum levels of sAPRIL & CEA were significantly elevated in those patients with advancing stages (C & D) compared with stages (A & B) and lower levels were found in patients who had surgical removal of tumor or received chemotherapy. Also a positive relation was found between sAPRIL & CEA with alcohol intake and smoking

Conclusion: according to this study sample it was found that sAPRIL and CEA together are strong indicators for colon cancer screening & diagnosis, and by this will reduce the need for more invasive screening & diagnostic tools.

Key words: colon cancer, tumor marker, CEA, sAPRIL.

Introduction:

Colonic cancer is a very common disease world-wide being fourth most common cancer characterized by abnormal proliferation of the epithelium of colon then taking full colon wall thickness then spread to the surrounding lymph nodes and tissues and finally distant metastasis. It is one of most complicated diseases with debilitating symptoms which becomes more severe, prominent and specific with advancing stage with high percent of fatality and relatively short survival if diagnosed late or if left untreated [1]. Therefore, finding an easy, non-invasive and readily available means (marker) for diagnosing colonic cancer would be of great value particularly if the same biomarker can be used for diagnosis, screening, prognosis and monitoring the efficacy of different treatment modalities. CEA is a protein found in many types of cells but associated with tumors and the developing fetus. Carcinoembryonic reflects the fact that CEA is made by some cancers "carcino-" and by the developing fetus "-embryonic [1]. CEA is tested in blood. The normal range is <2.5 nanogram (ng)/ml in an adult non-smoker and<5.0 ng/ml in a smoker Serum A proliferation-inducing ligand (sAPRIL) is a member of the tumor necrosis factor family that is known to bind to two receptors, B-cell maturation antigen (BCMA) and transmembrane activator and CAML interactor in addition APRIL can bind to heparan sulfate proteoglycans which act as a scaffold for proper signaling [2].

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Patients and methods:-

This study was applied on 35 patients (17 females & 18 males) with colon cancer their ages ranged 53-82 years old (6 patients received chemotherapy and 5 of them have undergone surgical removal of the primary tumor and 4 different patients received immunotherapy with 20 smokers and 4 alcoholic), compared to 35 patients (19 females & 16 males) with benign polyps their ages ranged 29-56 years old and 15 apparently healthy persons (7 females & 8 males) as a negative control their ages ranged 25-43 years. According to DUKE's staging: 6 colon cancer patients were stage A, 12 were stage B, 12 were stage C and 5 were stage D. [3] All individuals in this study (whom attended Baghdad Teaching Hospital of Gastroenterology and Hepatology hospital) were subjected to blood sampling for measuring their serum CEA and sAPRIL using Enzyme Linked ImmunoSorbent Assay (ELISA) technique which was done at Teaching Labratories /

Medical City (CEA kit: Monobind inc., U.S.A., 1825-300A) (sAPRIL kit: TW-reagent, China, 4008-723-722).

Statistical analysis:

Data expressed as mean ±SD. Statistical differences between the groups were determined according to ANOVA test and student t-test result considered to be Significant if P value <0.05, The difference in median between 2 groups was assessed by non-parameteric test (Mann-Whitney), while between three groups or more Kruskal-Wallis test was used.

Results:

This study results presented were based on the analysis of a random sample of 35 cases with a verified diagnosis of colon cancer. The cases group was compared to a random sample of 35 positive controls (Benign colon polyps) and 15 healthy control subjects. The mean age of cases group (69 years) was significantly higher than that of positive controls (43.5 years), which was higher than that of healthy controls (31.7 years). Serum levels of CEA & sAPRIL were higher in colon cancer patients than in both benign polyps and healthy controls as shown in table 1. The optimum cutoff value for CEA was 2.65 ng/dl with 100% sensitivity and 98% specificity, while sAPRIL optimum cutoff value was 123 ng/dl with sensitivity 100% & 98% specificity,for both when used to diagnose colon cancer as shown in table 2. Median serum levels of both CEA & sAPRIL are both higher with advancing stage and lower in patients received chemotherapy, immunotherapy and surgical operation as shown in tables 3&4.

Table 1: The difference in mean serum CEA and sAPRIL between the three study groups

	Study group			
	Healthy controls	Benign polyps	Ca Colon	P (Kruskal-Wallis)
Serum CEA (ng/ml)				<0.001
Range	(0.12 to 1.7)	(1.02 to 2.76)	(2.69 to 260.28)	
N	15	35	35	
Mean Rank	8.9	31.5	67	
P (Mann-Whiteny) for difference in median between:				
Ca Colon x Healthy controls <0.001				
Benign polyps x Healthy controls <0.001				
Ca Colon x Benign polyps <0.001				
Serum APRIL (ng/ml)				<0.001
Range	(51.85 to 82.44)	(85.98 to 180.24)	(123.16 to 403.08)	
N	15	35	35	
Mean Rank	8	33.7	67.3	
P (Mann-Whiteny) for difference in median between:				
Ca Colon x Healthy controls <0.001				
Benign polyps x Healthy controls <0.001				
Ca Colon x Benign polyps <0.001				

Table 1 Showing advanced ages are correlated with colon cancer more than younger ones this may be due to aging process, higher mutation chances in elderly and predisposion to mutagenic insults for longer time.

Table 2: Validity parameters for CEA and sAPRIL when used as test to diagnose Ca Colon differentiating it from controls (Benign + healthy).

				PPV at pretest probability =		
Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy	50%	90%	NPV at pretest probability = 10%
Serum CEA						
2.65 (Optimum cut-off)	100.0	98.0	98.8	98.0	99.8	100.0
2.8 (Highest specificity)	94.3	100.0	97.6	100.0	100.0	99.4
Serum sAPRIL						
123 (Optimum cut-off)	100.0	98.0	98.8	98.0	99.8	100.0
180.4 (Highest specificity)	25.7	100.0	69.0	100.0	100.0	92.4
Serial combination of both criteria at optimum cut-off value	100.0	100.0	100.0	100.0	100.0	100.0

Table 2 is showing that both CEA & sAPRIL tests are both sensitive and specific for screening and prognosis of colon cancer but routinely only CEA test is used prior to more invasive methods, now it seems that sAPRIL can support the CEA may be to reduce the need for more invasive tools of diagnosis and screening.

Table 3: The median serum CEA by selected explanatory variables among cases with Colon Ca.

	Serum CEA					
	Range	Median	Inter-quartile range	N	Mean Rank	P
DUKE's Staging						
Stage-A	(2.76 to 3.14)	2.99	(2.88 to 3.07)	4	6.5	<0.001
Stage-B	(2.95 to 7.83)	4.21	(3.6 to 5.06)	9	14.9	
Stage-C	(2.69 to 14.16)	4.98	(2.92 to 6.4)	12	14.3	
Stage-D	(7.52 to 260.28)	18.43	(11.57 to 54.75)	10	29.9	
r=0.70 P<0.001						
Chemotherapy						
Negative	(2.69 to 260.28)	6.56	(3.6 to 13.3)	26	20.6	0.01
Positive	(2.76 to 7.83)	3.14	(2.92 to 4.29)	9	10.4	
Surgery						
Negative	(2.69 to 260.28)	6.56	(3.6 to 13.3)	26	20.6	0.01
Positive	(2.76 to 7.83)	3.14	(2.92 to 4.29)	9	10.4	
Immunotherapy						
Negative	(2.76 to 260.28)	6.36	(3.53 to 12.59)	31	19.4	0.028
Positive	(2.69 to 3.6)	3.07	(2.84 to 3.37)	4	7.4	

Table 3 showing correlation between CEA and different parameters that affect progression of colon cancer and thus serum levels of its markers. Level of CEA is higher with advancing stage of colon cancer may be due to bigger size of tumor and by this more cells producing CEA. Other parameters are chemotherapy, immunotherapy and surgical removal are all showing levels of CEA lower in those patients undergone these procedures, this is because such treating options reduce tumor size and thus less cells producing serum markers.

Table 4: The median serum sAPRIL by selected explanatory variables among cases with Colon Ca.

	Serum sAPRIL					
	Range	Median	Inter-quartile range	N	Mean Rank	P
DUKE's Staging						
Stage-A	(123.16 to 127.96)	125.2	(123.29 to 127.46)	4	4	<0.001
Stage-B	(126.44 to 135.04)	130.5	(127.2 to 133.78)	9	9.2	
Stage-C	(130.49 to 198.01)	141.1	(137.57 to 158.43)	12	19.3	
Stage-D	(170.44 to 403.08)	202.1	(180.56 to 283.22)	10	30	
r=0.91 P<0.001						
Chemotherapy						
Negative	(123.16 to 403.08)	158.4	(134.03 to 182.83)	26	21.3	0.001
Positive	(123.41 to 139.09)	127.2	(126.8 to 133.52)	9	8.4	
Surgery						
Negative	(123.16 to 403.08)	158.4	(134.03 to 182.83)	26	21.3	0.001
Positive	(123.41 to 139.09)	127.2	(126.8 to 133.52)	9	8.4	
Immunotherapy						
Negative	(123.16 to 403.08)	139.1	(130.49 to 182.58)	31	18.8	0.19[NS]
Positive	(126.95 to 143.13)	131.0	(127.46 to 138.58)	4	11.8	

Table 4 showing correlation between sAPRIL and different parameters that affect progression of colon cancer and thus serum levels of its markers. Level of sAPRIL is higher with advancing stage of colon cancer may be due to bigger size of tumor and by this more cells producing sAPRIL. Other parameters are chemotherapy, immunotherapy and surgical removal are all showing levels of sAPRIL lower in those patients undergone these procedures, this is because such treating options reduce tumor size and thus less cells producing serum markers.

Discussion:

The present work attempted to study the advantage of using ELISA to detect some tumor markers (sAPRIL & CEA) in patients with colonic cancer and their benefit in diagnosis and screening of colon cancer, which is according to reference performed for the first time in our country. The current study showed that the mostly affected group with colonic cancer are those aged between (53-82) years of age (average 68.5±6.4 years of age), with higher values of serological markers in older patients compared to younger ages with ($P < 0.001$), this agreed with many other studies around the world [8, 9, 10, 11, 12]. The current study found highly significant association between CEA & sAPRIL levels in those with colonic cancer compared to benign colon polyps or healthy control being strong indicator together than each alone and this agrees with many other studies [13, 14] . Also a strong positive relationship found between CEA & sAPRIL and colon cancer stage with ($P < 0.001$). CEA & sAPRIL levels found to be lower in people who

received chemotherapy compared to those whom didn't with (P = 0.01 & 0.001 respectively), also patients whom underwent surgical removal of colonic malignant tumor showed lower levels compared to those didn't with (P = 0.01 & 0.001 respectively). Alcoholic patients showed higher levels of CEA & sAPRIL than non-alcoholic with (P = 0.003 & 0.001 respectively), all these agrees with many other studies [15, 16, 17, 18, 19, 20]. In conclusion, Serum sAPRIL and CEA were significantly higher in colonic cancer patients than in both benign polyps and negative controls, their levels were very useful in screening and prognosis of the disease, and the levels of sAPRIL & CEA appear to be significantly affected by different treatment options including surgical removal, chemotherapy and immunotherapy , also a strong correlation between sAPRIL & CEA levels with the stage of colonic cancer at time of the test was found to be significant.

Author contribution:

Study conception: Dr.Sarmad M.H. Zeiny
Study design: Dr.Sarmad M.H. Zeiny
Acquisition of data analysis: Dr. Aqeel Hatam Mahmood
Interpretation of data: Dr.Sarmad M.H. Zeiny & Dr. Aqeel Hatam Mahmood
Drafting of manuscript: Dr. Aqeel Hatam Mahmood
Data collection : patients whom undergone surgery or colonoscopy were operated by Dr.Aqeel Shaker Mahmood (as surgeon).

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