



COMPARISON BETWEEN TUMOR MARKER CEA, CA19-9 AND SOME MARKER ENZYMES (ALKALINE SPHINGOMYELINASE, CYCLOOXYGENASE-2, THYMIDYLATE SYNTHASE AND ARGINASE) IN SERUM OF COLON CANCER PATIENTS.

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ABSTRACT

Carcinoma of the colon was ranked as the sixth cancer among the top ten cancers in Iraq. Different markers are used for different purposes – namely, some of them are more appropriate for the follow-up of the disease and the others for the early detection of the disease recurrence. In many studies, an increase in CA 19-9 has been found to indicate a poor prognosis and high serum levels of either CEA or CA 19-9 in patients with colorectal cancer are significant, independent prognostic factors. **Method:** 104 patients diagnosed with colon cancer, period March 2013 to April 2014 from the patients treated in Mosul Oncology and Nuclear Medicine Hospital, Ten milliliters of venous blood was taken from each patient, then serum were separated for quantitative measurement of CEA and CA19-9 levels on mini VIDAS (Biomerieux, UK), Alk-SMase, COX2 and THYMS was determined

by using ELISA. Arginase enzyme activity was determined according to (Kocna *et al.*, 1996) procedure. **Results:** The statistical analysis of data listed in table (18) show that there was significant difference ($P < 0.05$) of enzymatic activity for serum (Cyclooxygenase-2, and Arginase) which significantly increase (26.77 ± 1.91 U/L, 23.3 ± 2.50 ng/ml) respectively for patients with early stage A colon cancer compared with controls (12.57 ± 1.65 U/L, 4.18 ± 0.34 ng/ml) where as statistical analysis shows non significant increase ($P > 0.05$) with the levels of CEA and C19-9 tumor marker (4.04 ± 1.6 ng/mL, 10.9 ± 2.1 U/ml) respectively compared with controls (2.8 ± 1.06 ng/mL, 7.2 ± 1.7 U/ml) measured in stage A revealed that

(Cyclooxygenase-2 and Arginase) may play a key role in the early stage of intestinal polyp-formation. This study also includes the measurement of the enzymatic activity of serum alkaline sphingomyelinase which was significantly decreased ($P < 0.05$) in colon cancer patients (82.21 ± 6.95 U/L) in stage A compared with controls (117.10 ± 6.25 U/L) and this decrease was independent of Dukes stage, thus strengthening the hypothesized validity of this assay to be used as a serum test for the early detection of colonic neoplasia. As table (18) shows that serum TYMS activities in early stages A, (54.93 ± 25.60 U/L), were non-significantly increased compared with healthy control (44.22 ± 18.26 U/L). **Conclusions:** this study shows that these antigenic tumor markers are not sensitive for early colon cancer detection. But these parameters may be used as a prognostic indicator to predict the aggressiveness of the malignant tumor in colon cancer.

KEYWORD: CEA, CA19-9, Serum Alkaline Sphingomyelinase (Alk-SMase), Human Cyclooxygenase-2(COX2), Serum thymidylate synthase (THYMS) and Arginase, colon Cancer.

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues (Hasan *et al.*, 2013; Dzivenu *et al.*, 2003). Large intestine performs wide variety of functions, ranging from breakage of large molecules to nutrients and water absorption (Rathore *et al.*, 2013) Colon is one major constituent of large intestine, and its cancer is a major reason of deaths in western and industrialized world (Kaur *et al.*, 2013; AL-Hasnawi *et al.*, 2009). Colorectal cancer is the second most common cause of cancer related death in western countries after lung cancer in men and breast cancer in women (Mihajlovic-Bozic, 2004). Carcinoma of the colon was ranked as the sixth cancer among the top ten cancers in Iraq (M. O. H.; 2009). Colorectal cancer can be classified by a system called Dukes' staging ranging from stage A to stage D, In 1929 Cuthbert Dukes proposed a classification designed to represent a step-wise progression of local-regional invasion by rectal cancers. The classification has been modified on many occasions in an attempt to increase its prognostic value. The most commonly employed modification of Dukes' system is that of Astler and Coller.(Adachi *et al.*,1994). The Dukes' stage describes the extent of invasion or spread of a tumor and correlates with overall survival, i.e. patients have an 83% survival chance with a Dukes stage A tumor versus 3% chance of survival if diagnosed with Dukes' stage D, both over five years (Campbell *et al.*, 2001; Brünner *et al.*, 2006) Tumour

markers are substances, usually proteins that are produced by the body in response to cancer growth or by the cancer tissue itself. These substances may be detected in blood, urine and tissue samples. Some tumor markers are specific for a particular type of cancer, while others are seen in several cancer types (Hamed *et al.*, 2011). The qualitative and quantitative evaluation of these markers is possible through modern techniques of sensitive immunoassays using monoclonal or polyclonal antibodies or polymerase chain reaction techniques (Tietz, 2006; Selvam, 2011). Different markers are used for different purposes – namely, some of them are more appropriate for the follow-up of the disease and the others for the early detection of the disease recurrence (Bast *et al.*, 2001). In body fluids, tumor markers are found in low concentrations and for their determination highly sensitive technology is needed. The techniques that are being used are more or less based on the same principle – *i.e.* the determination of antigen-antibody complexes. Most widely used techniques are the radio-immune assay, the enzyme-immune assay, and the luminometric-immune assay, which differ in the compound bound to the detection antibodies, and in the method of detection of the formed complexes (Novaković, 2004). Carcinoembryonic antigen (CEA) is a high molecular weight glycoprotein belonging to the immunoglobulin super family. The carboxy-terminal of CEA contains a hydrophobic region which is modified to provide a glycosyl phosphatidyl inositol link to the cell membrane (Tanaka *et al.*, 2010). While the presence can be determined in biopsy samples, it is usually identified in serum. This protein has been used for many years as a biomarker of CRC as well as cancers developing in other tissues (CEA) was first described in 1965 by Gold and Freedman and it is most widely used tumor markers worldwide (Al-Saadi *et al.*, 2014). CA 19-9 is synthesized by normal human pancreatic and biliary ductular cell and by gastric, colon, endometrial and salivary epithelia (Pavai, 2003). In serum, it exists as a mucin, a high molecular mass (200 to 1000 kDa) glycoprotein complex. Elevations in CA 19-9 level correlate with the degree of tumor differentiation as well as the extent of tumor mass (Abdallah *et al.*, 2013). Carbohydrate antigen 19-9 (CA 19-9) is a ligand for E-selectin that plays an important role in the adhesion of cancer cells to endothelial cells. It has been used as a tumor marker in gastrointestinal cancers. It may also be increased in several benign diseases. In many studies, an increase in CA 19-9 has been found to indicate a poor prognosis and high serum levels of either CEA or CA 19-9 in patients with colorectal cancer are significant, independent prognostic factors (Sisik *et al.*, 2013). American Society of Clinical Oncology guidelines suggested that serum testing for CA 19-9 is an integral part of the diagnosis and management of colorectal carcinomas. Sphingomyelinase is an enzyme that was first discovered in the intestinal tract by Nilsson in 1969 (Duan, 2006). It has now received real attention

only in the last decade, when dietary sphingomyelin was found to inhibit colonic tumorigenesis in animals. and the first mutation of the enzyme was discovered in 2004 in one human colon cancer cell line(Wu *et al.*, 2004). Alkaline sphingomyelinase is present in the intestinal tract and additionally human bile. It hydrolyses sphingomyelin in both intestinal lumen and the mucosal membrane in a specific bile salt dependent manner. Several isoforms of alk-SMases have been identified and classified by their pH optima: alk-SMase has been located specifically to the intestinal tract, where high levels are found in the small intestine and lower in the colon with a gradual decline towards rectum, acid SMase and neutral SMases have been found in many tissues and are considered as common cellular enzymes (Wu *et al.* 2010; Kurek *et al.*, 2013). The enzyme shares no structural similarity with other SMases (Duan *et al.*, 2003). The enzyme is of specific properties, such as bile salt dependency, high stability, and tissue specific expression (Cheng *et al.*, 2002; Wu *et al.*, 2004). In the colon, the enzyme may play antiproliferative and anti-inflammatory roles through generating ceramide (Duan *et al.*, 2007; Wu *et al.*, 2006). COX-1 and COX-2 are the two isoforms of cyclooxygenase, which convert arachidonic acid (AA) into several eicosanoids such as prostaglandin, thromboxanes and prostacyclin, which participate in several normal physiologic processes and inflammation (Urade, 2008). COX-1 and COX-2 share the same substrates, generate the same products and catalyze the same reaction using identical catalytic mechanisms (Zha *et al.*, 2004). Whereas COX-1 is constitutively expressed in most tissues, COX-2 is an inducible enzyme, stimulated by cytokines, growth factors, oncogenes or tumor promoters during inflammation or malignancy (Gallegoa *et al.*, 2007). In recent years, overexpression of COX-2 has been reported in a variety of cancers (Urade, 2008). COX-2 is induced by stimuli such as mitogens, cytokines, growth factors and tumor promoters, and has been elucidated to be involved in cancer development and pathogenesis (Rakesh *et al.*, 2014). COX-2-expressing interstitial cells accelerates colon carcinogenesis (Ota *et al.*, 2002). Thymidylate synthase (TS) plays a central role in the biosynthesis of thymidylate, an essential precursor for DNA synthesis see (voeller *et al.*, 2004; Bruni *et al.* 2002)., the inhibition of TS result in the cessation of cellular proliferation and growth (Rahman *et al.*, 2004). Several clinical studies have shown that TS protein and mRNA levels are higher in cervical, breast, kidney, bladder, lung, and gastrointestinal tumor tissues than in their normal counterparts and that high TS level have been associated with poor clinical outcome in these cancer (Nomura *et al.*, 2002; Mizutani *et al.*, 2003). Tumors with elevated TS levels are thought to undergo more progressive cellular proliferation, which in turn is associated with tumor invasiveness and metastasis (Mizutani *et al.*, 2003; Edler *et al.*, 2000). Due to the importance of arginase

enzyme in different malignant disorders Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) is homotrimeric binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine, rendering urea for ammonia elimination, mainly in urotelic animals, and L-ornithine (a non-protein amino acid) for biosynthetic pathways. There are at least two forms of arginase. Arginase cytosolic and is most abundant in the liver, primarily responsible for ammonia detoxification as urea (Mahmoud *et al.*, 2009). A second isoenzyme, arginase II, is involved in the production of ornithine as a precursor to proline, glutamate or polyamines, such as spermine and putresine, essential for cellular growth (Ash, 2004). Polyamines are vital for cell proliferation and it is possible that the increased level of ornithine, due to the elevated arginase activity, may be linked to the development of carcinogenesis (Soda, 2011). The enzyme also is present in other human tissues, such as kidney, brain, lung, stomach, bowel, prostate lactating mammary gland and activated macrophages, where its physiologic role is still poorly understood (Munder, 2009). Arginase is a very stable enzyme and it does not lose its activity when it is stored at low temperatures for long periods of time. In a previous report, we showed that two extrahepatic arginases are present in human colon carcinoma and CRC, and only one of them appears in blood serum. Its activity is low in healthy individuals and rises significantly in the serum from patients with primary colorectal cancer (Porembaska *et al.*, 2002). Arginase activity is markedly elevated in prostatic carcinoma, gastric cancer, colorectal cancer, chronic lymphocytic leukemia (Konarska *et al.*, 1993). Non-small cell lung carcinoma and breast cancer. Increased arginase activity has also been reported in cancer metastases (Gökmen *et al.*, 2010).

METHOD

This study include (104) patients diagnosed with colon cancer and proved by colonoscopy and histopathological examination of biopsy. Sample selected consecutively over the period March 2013 to April 2014 from the patients treated in Mosul Oncology and Nuclear Medicine Hospital, Jumhory Hospital/Mosul and, Azadi Teaching Hospital/Kirkuk. Clinical diagnosis in each case was established according to the oncologist. All cases and controls were aged (21-85–years). The patients in the study were clinically and histologically diagnosed as a newly (stage A and B), advanced (stage C) and metastasis (stage D) colon cancer patient and free from other chronic diseases such as diabetes, hypertension, or other cardiac, liver and renal disease. Female cases were not pregnant or lactating, beside one hundred normal blood donor individuals had been used as controls that were free from cancer or chronic diseases. All patients and controls gave informed, written consent for participation.

Serum from these patients obtained before surgery. Ten milliliters of venous blood was taken from each patient and left for (15) minutes at room temperature for coagulation, then serum were separated by centrifugation at (3000 xg)for 10 minutes and divided in aliquot and kept frozen at (-20°C) for the assays, CEA and CA19-9 kits (Biomerieux, France) was assayed using ELFA technique (Enzyme Linked Fluorescent Assay). for quantitative measurement of CEA and CA19-9 levels on mini VIDAS (Biomerieux, UK) instruments using serum specimens. Serum Alkaline Sphingomyelinase (Biosource, Cat. No.MBS039905, USA), Human Cyclooxygenase-2(Biosource, Cat. No.MBS164164, USA) and Serum thymidylate synthase (Cusabio Biotech, Cat. No. MBS032123, USA) was determined by using ELISA (FLx800 Fluorescence Microplate Reader and FLx800 Microplate Washer, BioTek, USA) kit assayed according to the manufactured procedure. Create a standard curve by reducing the data using computer software (Excel/ office 2007).

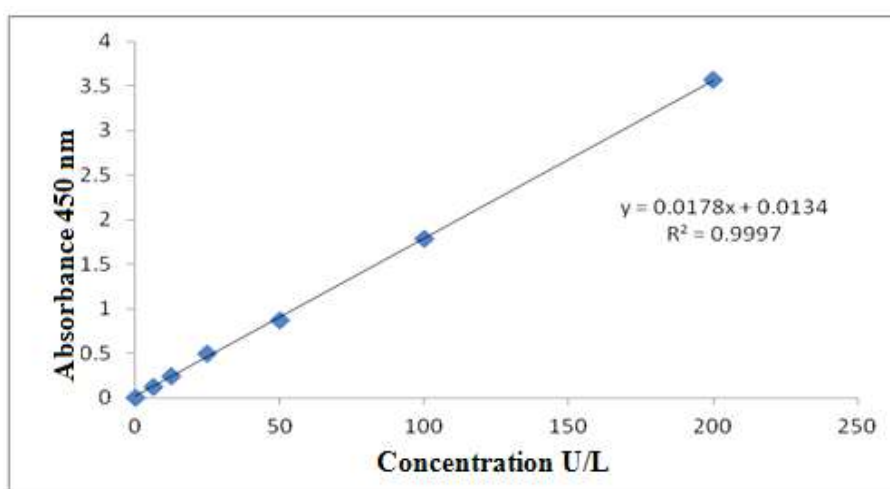


Figure (1) ALK-SMASE standard curve.

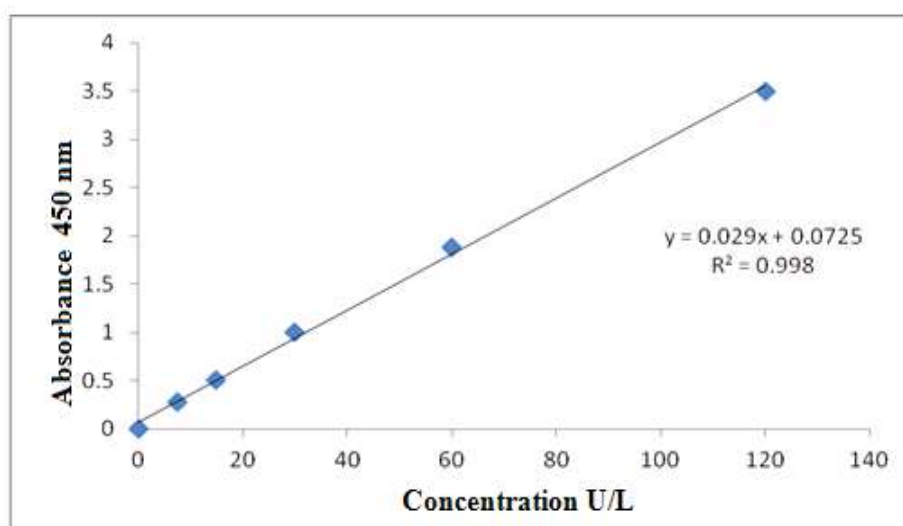
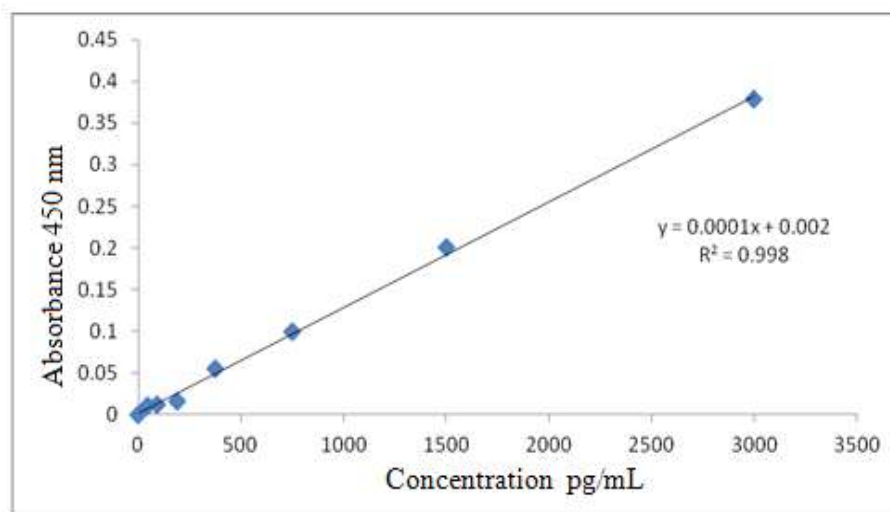
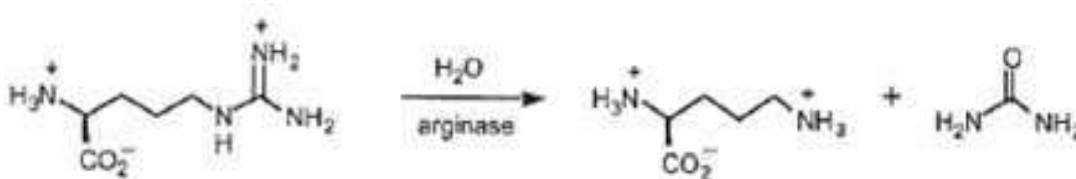


Figure (2) Human Cyclooxygenase-2 standard curve.**Figure (3) TYMS Standard curve.**

Arginase, (L-arginine amidinohydrolase, EC3.5.3.1.) The enzyme activity was determined according to (Kocna *et al.*, 1996) procedure. **Principle.** Arginase, (L-arginineamidinohydrolase, EC3.5.3.1) converts L-arginine into L-ornithine and urea. The elevated activity of arginase has been reported in serum as well as in colorectal, gastric and mammary carcinoma tissues. Arginase activity in the serum is determined by a two-step method. The concentration of ornithine (as a product of the first reaction) is measured by a colorimetric method (UV./VIS. Spectrophotometer, Cecil Instruments Ltd. GE, England).

**Figure (4) The arginase reaction(Ash, 2004).**

Reaction conditions consist: 500 μl of assay solutions (35 mmol/l Tris-HCl buffer pH 9.5 - 20, arginine 0.348, 0.009 gm MnCl_2), 25 μl of serum, 25 μl of 5 mmol/l Tris-HCl buffer. The incubation of samples was carried out in a water-bath at 37°C for 120 minutes and stopped by immersing tubes in a boiling water-bath for 5 minutes concentration was determined by an end-point ninhydrin. Ninhydrin reagent (0.5 ml) and acetic acid (1.5 ml) were added to the incubation medium reaction carried out in a boiling water bath for 60 minutes. This reaction was stopped by cooling to room-temperature and the colored reaction product was evaluated by using a spectrophotometer at 515 nm in a 1 cm glass cuvette

ornithine concentration was calculated from calibration standards curve as shown in figure (5).

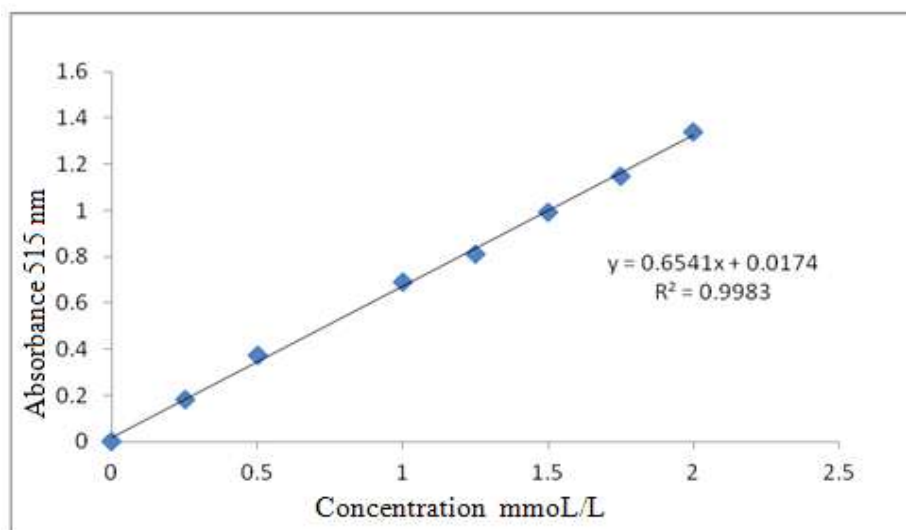


Figure (5) Ornithine - standard curve.

RESULTS AND DISCUSSION

Serum concentration of CEA in colon cancer patients.

Table (1) describes the results of the serum CEA concentrations in colon cancer patients with different Dukes Stage of the disease compared with Control.

Table (1): Serum CEA concentrations in colon cancer patients with different Dukes stage of the disease compared with control.

| CEA (ng/mL) | Dukes Stage M±SD | | | | Control M±SD |
|---------------|------------------|-----------|--------------------|-------------------|--------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 4.1±1.64 | 5.23±1.8 | 30.5±12.7 | 28.6±12.65 | 2.84±1.09 |
| Female | 3.8±1.8 | 4.8±2.2 | 28.2±12.6 | 27±11.2 | 2.76±1.09 |
| Total | 4.04±1.6 | 5.07±1.90 | 29.45±12.6* | 27.9±11.7* | 2.8±1.06 |

(*):Statistically significant differences at ($p < 0.05$) with control.

The results of the serum CEA concentrations in colon cancer patients with different Dukes stage of the disease compared with control as shown in table (1) revealed a significant increase at probability ($p < 0.05$) in CEA Level in stages C and D which were, (29.45±12.6) ng/ml, (27.9±11.7) ng/ml respectively compared with control group (2.8±1.06) ng/ml. while there were no significant difference in serum CEA Level in stages A and B (4.04±1.6) ng/ml, (5.07±1.90) ng/ml respectively compared with control groups. These results had been accepted with several other researches such as (Polat *et al.*, 2014) who indicate that serum

CEA levels were significantly higher in stages C and D than in stage A. (Ding *et al.*, 2014) reported that the mean of serum's CEA level were higher in dukes stage C of tumor than stage B or A. (Eleftheriadis *et al.*, 2009) found a low sensitivity associated with serum CEA assays in the early stage detection of colorectal cancer's. While (Ding *et al.*, 2014) showed that CEA level can be different between Duke stage A and C, C and B, but not between A and B. (Al-bayatti, 2009) indicate that the elevation of CEA level occur only in patients with advanced stages in colon cancer. Serum carcino embryonic antigen (CEA) is a 201 kDa highly glycosylated antigen expressed on the apical surface of colonic epithelial cells and excreted via the colonic lumen (Elias *et al.*, 2012). With the disruption of normal tissue architecture in malignancy and loss of polarization of neoplastic cells located deep inside the tumor glandular tissue, CEA may be expressed on the whole cell surface and is eventually shed to the bloodstream leading to serum CEA level's rise (Tibbetts *et al.*, 1993; Hammarstrom, 1999).

Results in table (12) also shows that there is no significant differences in serum CEA level between males and females patients for each stage which was agree with (Mohammadi *et al.*, 2013) results who reported that no differences were observed between male and female patients and between patients aged <50 and >50 years regarding the distribution of serum CEA levels and CEA levels may rise in patients without recurrence, and may even be below the cut-off point of 5 µg/l in patients with disseminated disease. Therefore, additional biological markers are needed to improve the identification of patients at risk of recurrent disease after intended curative resection and to monitor such patients during and after adjuvant therapy (AL-Dulamy *et al.*, 2010).

Serum concentration of C19-9 in colon cancer patients

Table (2) describes the results of the serum CA 19-9 concentrations in colon cancer patients with different Dukes stage of the disease compared with control.

Table (2): Serum CA 19-9 concentrations (U/ml) in colon cancer patient with different Dukes stage of the disease compared with control.

| CA19-9 (U/ml) | Dukes Stage M±SD | | | | Control M±SD |
|---------------|------------------|---------------------|-------------------|------------------|--------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 11.4±2.32 | 55.5±5 | 108.4±7.4 | 92±7.1 | 7.2±1.54 |
| Female | 10.02 ±1.78 | 54.3±4.9 | 106±9.2 | 91±6.01 | 7.1±1.87 |
| Total | 10.9±2.18 | 55.08 ±4.87* | 107.3±8.3* | 91.6±6.8* | 7.2±1.68 |

(*):-Statistically significant differences at (p< 0.05) with control.

Results in table (2) revealed statistically significant increase at ($P < 0.05$) in the mean values of the serum's CA19-9 Level in patients with stages (B,C) and (D) (55.08 ± 4.8) U/ml, (107.3 ± 8.3) U/ml and (91.6 ± 6.8) U/ml respectively compared with controls (7.2 ± 1.68) U/ml, where as no significant variation in patients with stage (A) serum's CA19-9 (10.9 ± 2.18) U/ml compared with control group (7.2 ± 1.7) U/ml. These results indicate that CA19-9 concentration is significantly elevated in patients with metastatic disease and with increasing the degree of dysplasia or with the size of the lesion. This will agrees with the reports of (Pavai and Yap., 2003) who observed that CA19-9 was very high only in patients with advanced stages of colorectal carcinoma, and (Partyka, 2014) that reported increased serum CA19-9 Level in 21-67% of advanced colon cancer patients (Hyeon *et al.*, 2013), indicates that metastases showed a stronger expression of CA 19-9 than primary tumors and showed a lower expression in Dukes' A and B tumors than in more advanced stages. Beside (Narimatsu *et al.*, 1998) showed that the increasing of serum CA19-9 concentration is associated with a modification of the antigen expression. The values of CA19-9 in tissues are highest than values in the patients sera with the same type of cancer (Kajiwara *et al.*, 2005). This is an indicator that the cancer cells produce the antigen CA19-9 and release it to the blood (Al-dujaili *et al.*, 2009).

The activity of some marker enzymes in serum of colon cancer patients.

Serum Alkaline Sphingomyelinase (alk-SMase). Activity in colon cancer patients.

Table (3): Serum sphingomyelinase activity in different Dukes stages of colon cancer patients compared with control.

| Alk-SMase (U/L) | Dukes Stage M \pm SD | | | | Control M \pm SD |
|-----------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|--------------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 82.52 \pm 7.2 | 79.73 \pm 6.2 | 75.33 \pm 4.91 | 71.25 \pm 5.02 | 117.97 \pm 6.88 |
| Female | 81.6 \pm 7.1 | 79.5 \pm 5.5 | 75.56 \pm 3.34 | 71.66 \pm 5.64 | 116.24 \pm 5.64 |
| Total | 82.21\pm6.95* | 79.66\pm5.81* | 75.44\pm4.22* | 71.44\pm5.19* | 117.10 \pm 6.25 |

(*):-Statistically significant differences at ($p < 0.05$) with control.

Serum alk-SMase activities related to Dukes stage of colon cancer were shown to be significantly lower in colon cancer patients compared with healthy control, as table (3) revealed that. Serum, alk-SMase activities was found to be significantly reduced in the early stages A, B stages which were (82.21 ± 6.95) U/L and (79.66 ± 5.81) U/L respectively compared with healthy (117.10 ± 6.25) U/L). In addition reduced serum, alk-SMase activities was shown in advanced and metastasis stages C, D which were (75.44 ± 5.19) and (71.44 ± 5.19) U/L respectively compared with healthy (117.10 ± 6.25) U/L), this agrees with

the reports of (Hertervig *et al.*, 1997), who found that, alk-SMase activity preferentially decreases in human colorectal carcinoma, suggesting a regulatory role of the enzyme in colon mucosa cell proliferation. In addition (Hertervig1 *et al.*, 1999) showed that the analysis of, alk-SMase activity in intestinal biopsies from control and colorectal adenocarcinoma patients showed a significant decrease between the enzyme activity in tumor samples and controls with a mean reduction of 90%.

It is important to recognize that sphingomyelin pathway is considered as one of the most important intracellular mechanism in regulating cell-growth, differentiation and apoptosis (Condorelli *et al.*, 1999). Alk-SMase (no EC number assigned), is specifically expressed in the intestinal tract and human liver.

In the intestinal tract, alk-SMase may prevent colonic tumorigenesis. First, the enzyme generates antiproliferative and proapoptotic lipid messenger ceramide (Duan *et al.*, 2003). Second, the enzyme inhibits proliferation of human colon cancer cells (Hertervig *et al.*, 2003). Third, the enzyme activity is reduced in both long standing ulcerative colitis and colonic tumorigenesis (Sjöqvist *et al.*, 2002).

The results in table(3) also shows that no statistically significant difference was found between Dukes' stages B, C, and D, this agrees with (Duan *et al.*, 1997) who found that In relation to Dukes' stage of adenocarcinoma and tumor cell differentiation, the, alk-SMase activity in tumor samples was shown to be always significantly reduced when compared with normal mucosa, whereas no significant difference was observed when tumor samples, at all Dukes stages or differentiation grade, were compared. In addition (Marzio *et al.*, 2013) showed that, alk-SMase was significantly decreased in tumor line intestinal mucosa of patients compared with controls in dependently of Dukes' stage and tumor differentiation grade. These results suggest that the decreasing activity gradient from ascending colon to rectum could be totally abrogated in adenocarcinoma patients. Markedly reduced mucosal, alk-SMase activity has been associated with colorectal carcinoma (Hertervig *et al.*, 1997), colorectal adenomas (Duan, 2006).

Serum Cyclooxygenase-2 (COX2) (EC1.14.99.1), activity in colon cancer patients.**Table (4): Serum COX2 activity in different Dukes stages of colon cancer patients compared with control.**

| COX2 (U/L) | Dukes Stage M±SD | | | | Control M±SD |
|--------------|--------------------|--------------------|--------------------|--------------------|--------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 27.24±1.6 | 28.6±1.3 | 30.1±2.7 | 27.04±1.6 | 12.07±1.09 |
| Female | 25.9±2.2 | 27.3±1.8 | 30.2±2.04 | 27.5±1.3 | 12.78±2.02 |
| Total | 26.77±1.91* | 28.05±1.60* | 30.18±2.42* | 27.26±1.56* | 12.43±1.65 |

(*):-statistically significant differences at ($p < 0.05$) with control.

The mean of COX2 activity was found to be significantly increased in serum of malignant colon cancer in comparison to healthy control serum. as table (4) revealed. Serum COX2 activities in early stages A, B, (26.77±1.91) U/L, (28.05±1.60) U/L respectively were significantly increased compared with healthy control (12.43±1.65) U/L. In addition significantly increased in mean activity of COX2 was shown in advanced and metastasis stages C, D which were (30.18±2.42) U/L and (27.26±1.56) U/L respectively compared with healthy (12.57±1.68) U/L, the results was similar to (Singh *et al.*, 2011) who reported that the serum COX-2 was found to be significantly ($P > 0.0001$) elevate in breast and oral cancer patients compare to the normal control. However, the results in table (4) shows the relationship between COX2 activity levels and tumor stages in patients with advanced stage (C) showed higher COX2 activity compared to those with early stage (A, B) which in agreement with (Feng, 2007) who reported that gastric cancer and colorectal cancer in patients with advanced serum COX-2 levels were significantly higher. In addition the differences are also further in patients with stage D showed lower activity levels when compared with stage B and C. These findings support the evidence that Aspirin use was associated with a risk reduction in patients whose colon tumors expressed higher levels of COX-2 and this agreement with (Chan *et al.*, 2007) Figure (29). In addition to prevention, regular aspirin use after the diagnosis of CRC at stage A, B and C improves overall survival, especially among individuals with tumors that over express COX-2 (Wang and Bois., 2010), suggesting the potential therapeutic use of non-steroidal anti-inflammatory drugs (NSAID), such as aspirin and ibuprofen in advanced CRC.

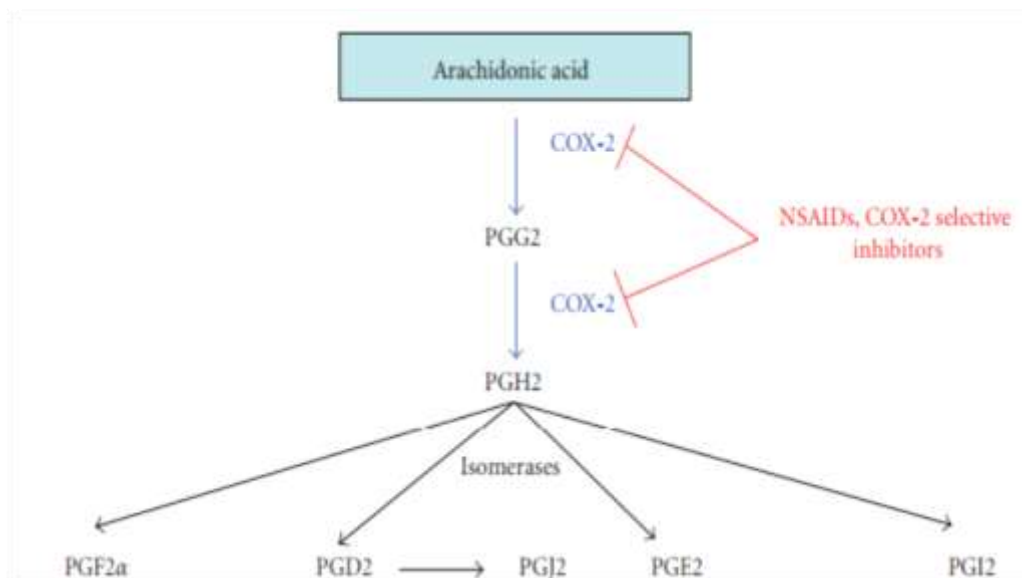


Figure (6): Metabolism of arachidonic acid by cyclooxygenase-2.

Thymidylate Synthase: (TYMS) (5,10-methylenetetrahydrofolate, dUMP C methyltransferase (EC2.1.1.148).

Table (5): Serum thymidylate synthase activity in different Dukes stages of colon cancer patients compared with control.

| TYMS (U/L) | Dukes Stage M±SD | | | | Control M±SD |
|------------|------------------|-------------|---------------|---------------|--------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 48.58±18.59 | 51.69±22.47 | 139.59±30.77 | 135.86±16.83 | 49.06±22.06 |
| Female | 48.8±18.94 | 53.89±19.94 | 120.18±24.41 | 128.08±19.87 | 39.52±13.79 |
| Total | 54.93±25.60 | 52.57±20.78 | 130.64±29.43* | 132.32±18.26* | 44.22±18.81 |

(*):-statistically significant differences at ($p < 0.05$) with control.

Serum Thymidylate Synthase TYMS Activity was found to be significantly increased in serum of colon cancer patients in comparison to healthy control. As table (5) show that serum TYMS activities in early stages A, B, (54.93±25.60 U/L), (52.57±20.78 U/L) were nonsignificant increased compared with healthy control (44.22±18.26U/L). In addition significantly increased in serum TYMS activities in advanced and metastasis stages C, D which were (130±29.43U/L) and (132.32 ±18.26U/L) respectively compared with healthy control (44.3±17.5U/L), the results was agree with (Patla and Pawlga, 2005) observed that TYMS expression in the metastasis would be different than in the primary tumor, which could determine the response to 5-FU based chemotherapy.

Serum concentration of Arginase (L-arginine amidino hydr-olase, EC3.5.3.1.), in colon cancer patients.

Table (6): Serum arginase concentrations in different Dukes stages of colon cancer patients compared with control.

| Arginase (ng/ml) | Dukes Stage M±SD | | | | Control M±SD |
|------------------|-------------------|-----------------|-----------------|------------------|--------------|
| | Dukes A | Dukes B | Dukes C | Dukes D | |
| Male | 23.8±2.7 | 9.9±0.96 | 8.3±1.4 | 11.2±1.27 | 4.15 ±0.34 |
| Female | 22.4±1.9 | 9.8 ±0.94 | 8.24±1.8 | 11±0.97 | 4.22±0.36 |
| Total | 23.3±2.50* | 9.9±0.9* | 8.3±1.4* | 11.1±1.1* | 4.18±0.35 |

(*):-statistically significant differences at ($p < 0.05$) with control.

In the present study, the activity levels of arginase were determined in colon cancer patients serum that revealed significantly increased levels of arginase in colon cancer patients compared to normal subjects ($P < 0.05$). The mean activity of arginase was found to be significantly higher in the early stages A (23.3 ± 2.50), ($P < 0.05$), and in the advanced stages B, C, and D which was (9.9 ± 0.9), (8.3 ± 1.4), (11.1 ± 1.1) respectively for colon cancer patients in comparison with those of the normal subjects. (4.18 ± 0.35). table(17) shows that there is no significant differences in mean activity of arginase between males and females patients for each stage and control. Investigators observe either enzymes that are native to normal tissue or those that could be associated with changes in metabolism and that are unique to cancer tissue. One of these enzymes is arginase. The enzyme has at least two forms. (Arginase I) is cytosolic and is most abundant in the liver, primarily responsible for ammonia detoxification as urea. A second isoenzyme, (arginase II), is involved in the production of ornithine which acts as a precursor to proline, glutamate or polyamines, such as spermine and putresine, essential for cellular growth (Cederbaum, 2000). Polyamines are vital for cell proliferation and it is possible that the increased level of ornithine due to the elevated arginase activity may be linked to the development of carcinogenesis (Porembaska *et al.*, 2003). Leu and Wang 1991 determined that the serum arginase activity had been 2 times higher in the colorectal cancer tissues in colorectal cancer patients when compared to normal mucosal tissues.

The present data are in agreement with previous studies (Leu and Wang, 1991; Porembaska *et al.*, 2003) who reported that serum arginase activity levels in patients with colon cancer significantly higher than those found in control subjects. The same researchers also investigated the relationship between serum arginase activity and the stage of gastric cancer. The mean serum arginase activity in patients with early stage gastric cancer was significantly higher than the control group. Moreover, serum arginase activity was higher in the advanced stage of gastric cancer than in the early-stage gastric cancer and in the control group. Previous studies, determined that arginase activity was found to have a close association with

various types of cancer, especially colorectal, prostate, pancreas and stomach cancer, and increased activity has been also demonstrated. The pattern of enzymatic alterations may be linked with the malignant state and the progression of cancerous cells in the tumor (Jamshidzadeh *et al.*, 2001). Differences in the activities or concentration of certain enzymes between cancer cells and their normal counterparts might be useful as biological markers of malignancy and/or aggressiveness in particular tumors (Tietz, 2006).

Comparison between patients in stage A colon cancer and control for measured (CEA, CA19-9, Alk-SMase, COX2, THYMS and Arginase)

The slow and progressive nature of colon cancer presents an opportunity to implement screening programs and diagnostic tools for the early detection of the disease that have the attempt to reduce incidence and to detect the disease in its early stages before symptoms are evident (Burt *et al.*, 2013). Due to the heterogeneous nature of CRC, a single biomarker is unlikely to have sufficient sensitivity or specificity for use as a stand-alone diagnostic screening test and a panel of markers may be more effective (Fung *et al.*, 2015).

Table (7) Comparison between patients in stage A colon cancer and control.

| Parameters | Mean \pm SD | |
|----------------|-----------------------------------|-------------------|
| | Duke stage A | Control |
| CEA ng/mL | 4.04 \pm 1.6 | 2.8 \pm 1.06 |
| CA19-9 ng/mL | 10.9 \pm 2.18 | 7.2 \pm 1.68 |
| Alk-SMase U/L | 82.21\pm6.95* | 117.10 \pm 6.25 |
| COX2 U/L | 26.77\pm1.91* | 12.43 \pm 1.65 |
| THYMS U/L | 54.93 \pm 25.60 | 44.22 \pm 18.81 |
| Arginase ng/ml | 23.3\pm2.50* | 4.18 \pm 0.35 |

(*):-statistically significant differences at ($P < 0.05$) with control.

The statistical analysis of data listed in table (7) show that there was significant difference ($P < 0.05$) of enzymatic activity for serum (Cyclooxygenase-2, and Arginase) which significantly increase (26.77 \pm 1.91 U/L, 23.3 \pm 2.50 ng/ml) respectively for patients with early stage A colon cancer compared with controls (12.57 \pm 1.65 U/L, 4.18 \pm 0.34 ng/ml) where as statistical analysis shows non significant increase ($P > 0.05$) with the levels of CEA and C19-9 tumor marker (4.04 \pm 1.6 ng/mL, 10.9 \pm 2.1 U/ml) respectively compared with controls (2.8 \pm 1.06 ng/mL, 7.2 \pm 1.7 U/ml) measured in stage A revealed that (Cyclooxygenase-2 and Arginase) may plays a key role in the early stage of intestinal polyp-formation. This study also include the measurement of the enzymatic activity of serum alkaline sphingomyelinase which was significantly decreased ($P < 0.05$) in colon cancer patients (82.21 \pm 6.95 U/L) in

stage A compared with controls (117.10 ± 6.25 U/L) and this decrease was independent of Dukes stage, thus strengthening the hypothesized validity of this assay to be used as serum test for the early detection of colonic neoplasia. As table (18) show that serum TYMS activities in early stages A, (54.93 ± 25.60 U/L), were non significant increased compared with healthy control (44.22 ± 18.26 U/L).

CONCLUSIONS

Results of this study conclude

1. Serum's CEA and CA19-9 concentrations revealed a significant increase at $p < 0.05$ in colon cancer patients in stages C and D in comparison with its concentration in control group. While no significant difference had been observed in stages A and B, there for this study shows that these antigenic tumor markers are not sensitive for early colon cancer detection. But these parameters may be used as a prognostic indicator to predict the aggressiveness of the malignant tumor in colon cancer.
2. Serum's enzymes such as thymidylate synthase, alkaline sphingomyelinase, cyclooxygenase-2 and arginase reflect the human intestinal mucosal enzyme level and could represent a new marker for human colorectal adenocarcinoma. Mainly taking into account its early appearance in intestinal neoplasms and may be useful for the early diagnosis of patients who develop recurrent colon cancer and colorectal liver metastases.
3. A significant increase was also noticed in additional enzymetic markers such as (Alkaline phosphatase, lactate dehydrogenases and α -amylases) compared with control group. This could be explained by the fact that the presence of the enzyme in high concentration inside the cell and the changes in the membrane permeability of the tumor cells would lead to the release of the enzyme into the systemic circulation. and it could be good indicators for early detection of colon cancer.

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