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RESEARCH ARTICLE

INSTITUTE OF GENETIC ENGINEERING AND BIOTECHNOLOGY FOR POST GRADUATE STUDIES

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ABSTRACT

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States. Iraq represent the sixth country incidence rates of colorectal cancer in Asia while India the first country followed by, Yemen, Bangladesh, Egypt and Pakistan. The purpose of presence study is the evaluation of the arginase activity of Iraqi Patient with colorectal disease and other intestinal disease in comparison with other colorectal cancer tests such as CEA, CA 19-9 in Iraqi patients and if it can be helpful in the diagnosis of patients with colorectal cancer and other intestinal disease. The activity of arginase was determined in blood serum was collected from 57 patients included 2 group of patients with colorectal cancer and other intestinal disease, in addition to The control group consisted of 20 healthy blood donors and compared with the level of CEA and CA 19-9. There is significant increase in arginase concentration in all groups versus the control group ($P<0.05$). CEA levels showed a significant increase only in colorectal cancer and inflammation versus the control group but there is no increase in the levels of in CEA in ulcerative and polyp groups versus the control group ($P<0.05$). We found the higher range for arginase, CEA and CA19-9 was in left colon rather than right colon and rectum.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States. In high-risk areas, such as Europe, the rates are gradually increasing. The incidence tends to be low in Africa and Asia and intermediate in southern parts of South America [(1)]. Iraq represent the sixth country incidence rates of colorectal cancer in Asia while India the first country followed by, Yemen, Bangladesh, Egypt and Pakistan [(2)]. Tumor markers are used clinically for diagnosis, staging, and monitoring of the disease. They are proteins released from dying tumor cells or produced by neoplastic cells [(3)].Great effort has been dedicated to the search of sensitive and specific markers of the disease and up to now, carcinoembryonic antigen (CEA) and the gastrointestinal cancer- associated carbohydrate antigen (CA 19-9) are the most widely applied markers in gastrointestinal malignancies, *e.g*., CRC or pancreatic cancer [(4,5)]. Determination of arginase activity as well as L-arginine concentration in blood serum may be useful in diagnosis of patients with colorectal cancer and colorectal cancer liver metastases[(6)]. Arginase (L-arginine amidinohydrolase, enzyme code number 3.5.3.1) is the final enzyme of the mammalian urea cycle, it catalyzes the hydrolysis of arginine to urea and ornithine [(7)]. There are two arginase isoenzymes encoded by distinct genes in mammalian tissues : liver-type arginase called arginase AI (cytosolic isoform) and extrahepatic type arginase called AII (mitochondrial isoform) Arginase AII is present in various tissues such as kidney, small intestine, stomach, pancreas, brain, and also in liver. In human liver arginase AI (previously called "hepatic arginase") is a key enzyme of the urea cycle and occurs in periportal hepatocytes, whereas arginase AII (so called "extrahepatic arginase") is localized in perivenous hepatocytes and its function is still poorly understood [(8)]. Besides urea, arginase takes part in the biosynthesis of ornithine this non -protein amino acid is a precursor for biosynthesis of the proline (for collagen), glutamate (for glutamine) and polyamines (for regulation of cell proliferation common substrate, arginine[(9)]. Arginase activity has been described in cancer and regeneration). Arginase also competes with nitric oxide synthase for the patients, it is thought to originate from tumor cells metabolizing arginine to ornithine needed to sustain rapid cell proliferation. Existence of suppressor myeloid cells producing arginase in human cancer patients. In addition, it supports the concept that blocking arginase may be an important step in the success of immunotherapy, because this lead to deplete of arginine which impair T cell and proliferation of T cells and cytokine production [(10)]. Simultaneous determination of L-arginine and arginase increases the value of arginase itself in diagnosis and follow up of patients with CRC and CRCLM[(11)]. There is important role for arginase in tumour immunology and many research indicate its potential role in the promotion of tumour growth via polyamine synthesis or down-regulation of NO-mediated tumour cytotoxicity. It also became clear that malignant tumours have evolved strategies to evade an effective tumour-cytotoxic immune response by inducing pathways of inflammation-associated immunosuppression. A key mechanism of tumour evasion from immune mediated destruction is the induced impairment of T cell functions [(12)]. The purpose of presence study is the evaluation of the arginase activity in comparison with other colorectal cancer tests such as CEA and CA 19-9 in Iraqis patients and if its can be helpful in the diagnosis of patients with colorectal cancer and other intestinal disease.

MATERIALS AND METHODS

Patients

The study groups included 57 patients who underwent to the Gastroenterology and Hepatology Diseases Center, and Teaching Laboratories between November, 2011 and May, 2012. The first group consisted of 35 patients with primary colorectal adenocarcinoma including 20 male and 15 female with a median age of 48.5 years range, (25–72). The first group of patients were classified as 2 patients with well differentiated tumor, 18 patients with moderately differentiated tumor according to the Modified

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Dukes classification of Astler and Coller,1954. 1 patient with villous tumor dysplasia and 14 colorectal cancer patients who diagnosed by physical. The second group consisted of 14 patients with intestinal disease (inflammation)7 male and 7 female with a median age 42.5 years range, (21–64). The third group included of 8 patients with (ulcerative and polyp) 3 male and 5 female, as diagnosed by colonoscopy , biopsy and confirmed by histopatholgist examination. Patients treated with radiotherapy or chemotherapy were excluded. The control group consisted of 20 healthy blood donors 9 females and 11males with a median age of 50 years, range (35–65 years). Blood samples were collected from Patients. In EDTA tube after fasting and the Plasma was separated by centrifugation at 3,000 x g for 10 min at room temperature. The Plasma was transferred to a clean tube and stored at -20 °C until use for the studies. The arginase level were estimated by ELISA Kit supplied from (Biocusa, China) . The CAE and CA19-9 concentrations were determined by ELISA Kit according to the methods described by manufactories (Human Diagnostic, German).The mean concentration of arginase 3.09 ± 0.22 ng/ml for control subjects according to[14]. Plasma CEA and CA19-9 concentrations were measured with CEA concentrations >7.0 ng/ml and CA19-9 concentrations >37 U/ml being regarded as elevated [15].

Statistical Analysis

The Statistical Analysis System- SAS (2004) was used to effect of difference factors in study parameters. The least significant difference –LSD test to the comparative between means in this study and Chi-square test was used to significant compare between percentage [16].

RESULTS AND DISCUSSION

The first researcher whose developed an efficient Enzyme Linked Immunosorbent Assay (ELISA) for measurement human liver type arginase in serum[17], this assay is highly sensitive specific and reproducible which able to determined arginase at concentrations low as several micrograms per liter without any prior processing of serum.

Table 1. Distribution of characteristics of the patients

Group	Male		Female		Age median	P Value (Chi-square-χ^2)
	No.	%	No.	%		
Colorectal cancer	20	57.14	15	42.86	48.5	0.0462 (3.97 *)
Inflammation	7	50.00	7	50.00	42.5	1.00 (0.00NS)
Ulcerative and Polyp	3	37.50	5	62.50	47.5	0.0027 (9.84 **)
Normal	11	55.00	9	45.00	2.86NS	2.86NS

* (P<0.05), ** (P<0.01), NS: Non-significant.

Table 2. Distribution & classification of tumour and type of disease

Group	Type	No.	Percentage (%)
Colorectal cancer	Well differentiated adenocacinma	2	3.51
	Moderately differentiated adenocacinoma	18	31.58
	Villous Tomuor	1	1.75
	Colorectal cancer	14	24.56
Inflammation	Inflammation	14	24.56
Polyp & Ulcerative	Polyp	3	5.26
	Ulcerative	5	8.78
Total	-------	57	100 %

In this study we determinate the arginase activity in serum of 57 patients with colorectal cancer, inflammation polyp and ulcerative by ELISA test, and compared our results with CEA and CA19-9. Table (1) show the distribution of characteristics of the patients**,** There is significant increase in patients with colorectal cancer and ulcerative, polyp. Patients versus the control group (P<0.05), (P<0.01) respectively but there is not significant increase in patients with inflammation and normal this indicate to lat diagnosis of disease. There is increase incidence of male patients against the female, which may be indicate that colorectal occur in male more than female due to the smoking and intake of alcohol.

While Table (2) show distribution & classification of tumour and type of disease, there is high percentage of tumour grade of Moderately differentiated adenocacinoma in colorectal cancer patients (31.58%). Also (24.56%) for patient with colorectal cancer who diagnosed by physical. Range, median and SE of the studied parameters in control versus patient groups (colorectal cancer, ulcerative, polyp and inflammation) as shows Table (3). There is significant increase in arginase concentration in all groups versus the control group (P<0.05).CEA levels showed a significant increase only in colorectal cancer and inflammation versus the control group but there is no increase in the levels of in CEA in ulcerative and polyp groups versus the control group (P<0.05), also, the result of CA19-9 demonstrated a significant increase in colorectal cancer, inflammation and polyp but no increase ulcerative versus the control group. The important thing is high percent (42.10%) of all patients did not express CA19. Arginase I-producing myeloid-derived suppressor cells deplete l-arginine (L-Arg) from the microenvironment, which arrests T cells in the G(0)-G(1) phase of the cell cycle.

This cell cycle arrest correlated with an inability to increase cyclin D3 expression resulting from a decreased mRNA stability and an impaired translation. this mechanisms leading to a decreased cyclin D3 mRNA stability in activated T cells cultured in medium deprived of L-Arg. These data contribute to an understanding of a central mechanism by which diseases characterized by increased arginase I production may cause T cell dysfunction [18]. Sidney and Morris [19] in their studies with cultured cells have demonstrated that arginine deficiency due to arginase can result in T cell dysfunction via reduced expression of the CD3zeta chain of the T cell receptor complex and cell cycle arrest, Immune cell dysfunction resulting from arginine deficiency is not limited to T cells but also can involve macrophages. In addition to its effect on NO production a recent study showed that arginine insufficiency also impairs the mitogen-activated protein kinase signaling pathway required for macrophage. Production of cytokines in response to bacterial endotoxin/lipopolysaccharide. Mielczarek *et al*., [20] showed that raised arginase activity was observed in serum of 85% of CRCM patients, whereas elevated levels of CEA and CA 19-9 were found in 63% and 42% of patients, respectively. The sensitivity test of arginase in colorectal cancer, inflammation and polyp and ulcerative where range (85.5%-75%) higher than CEA and CA19-9 range (51.1% -14.2),(65.7-30.7) respectively, while the specifity also was higher in arginase than CEA and CA19-9, it where (75%,14.2,14.2) respectively. In conclusion arginase was most sensitivity and specifity than CEA and Ca19-9, Table (4). There is significant differences in arginase activity were found between normal and pathologically changed mucosa, and between tissue with higher proliferation (cancer and inflammatory

Table (3): Arginase concentration , CEA & CA 19-9 in patients and controls

Type and patients No.	Arginase ng/ml		CEA ng/ml		CA19-9 U/ml	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Colorectal cancer (No.35)	9.99 ± 1.64	4.24- 38.99	31.89 ± 11.21	3.46- 250	41.69 ± 12.46	7.83- 324.95
Inflammation (N=14)	9.20 ± 2.66	3.09- 25.59	24.07 ± 20.56	3.66- 250	86.08 ± 44.14	11.31- 380.40
Ulcerative (N=5)	6.93 ± 1.99	3.21 -13.33	2.98 ± 1.04	2.61- 5.11	34.75 ± 32.52	9.13 -164.64
Polyp(N=3)	5.22 ± 1.27	3.52 -5.88	4.97 ± 1.10	3.66 -5.58	38.73 ± 18.74	0- 116.21
Normal (N=20)	1.77 ± 0.93	1.60- 2.97	4.21 ± 2.59	3.51- 5.21	6.42 ± 4.71	0- 15.88
LSD Value	6.406 *	---	22.370 *	---	29.72 *	---

* (P<0.05).

Table 4. Sensitivity and Specifity of Tumor Marker

Disease	Sensitivity Arginase	Sensitivity CEA	Sensitivity Ca-19-9
Colorectal cancer	85.7 %	51.1 %	65.7 %
Inflammation	75 %	14.2 %	30.7 %
Polyp and ulcerative	85 %	14.2%	28.5%
Specifity	75 %	14.2 %	14.2 %

Table 5. Tumor marker according to tumor site

Site of Tumor	Arginase Range (ng/ml)	CEA Range (u/ml)	Ca19-9 Range (u/ml)
Right colon	7.3-47.289	14.281-4.154	0-51.721
Left colon	17.41-2.449	95.191-2.589	0-144.513
Rectum	10.939-2.259	3.459-16.89	0-58.188

disease) and adenomatous polyps or post-resection mucosa in the large bowel [21]. The sensitivity, specificity, of preoperative serum CA19-9 levels were calculated as markers for recurrence by Park, *et al*., (2009), they found sensitivity and specificity were 23.6% and 88.6%, respectively. For preoperative CEA, the sensitivity and specificity were 38.8% and 83.5%, respectively [22]. Many studies showed the increase of arginase type liver in primary and metastatic colorectal cancer, Porembska *et al*., published that 94% for arginase activity and 64%,45% for CEA and Ca19-9 when they were determined Preoperative activity of arginase in blood serum of 86 patients with colorectal liver metastases and compared with the level of arginase CEA and CA 19-9. [23] and in inflammation and ulcerative[24]. Our investigation indicate there is a high number of patient did not express CA19-9. Even in those with large tumors, this is because of a deficiency of a fucosyltransferase enzyme that is needed to produce CA19-9 as well as the Lewis antigen[25]. Guidelines from The American Society of Clinical of Oncology (ASCO) discourage the use of CA19-9 as ascreening test for cancer, particularly pancreatic cancer. The reason is that the test may be falsely normal (false negative) in many cases, or abnormally elevated in people who have no cancer at all (false positive) [25,26]. Our results was lower level in CEA and Ca19-9 this may be due to clinical characteristic in patients.

When we comparable the data according to site of disease we found the higher range was in left colon rather than right colon and rectum for arginase ,CEA and CA19-9 as shown in table (5).CRCs are heterogeneous. More than a decade ago, it was proposed that two distinct categories of CRC existed based on tumor location (proximal or distal to the splenic flexure of the colon)[27]. Differences in physiology and anatomy, environmental carcinogens, genetic mechanisms, and prognosis between the three segments(left, right and rectum) support the 'three entities' concept[28]. High consumption of processed meat was associated with an increased risk of distal colon cancer but not of proximal colon cancer; association with red meat appeared to be stronger for rectal cancer than for colon cancer [29]. Asians and Pacific islanders are more susceptible to local distal colon and rectal cancers than Americans[30,31]. In conclusion we found that increase incident of colorectal in male other than female and, there significant increase in arginase level colorectal cancer, Inflammation polyp and ulcerative. The expression of CA19-9 was low level of high rate in Iraqi colorectal cancer patient. Arginase was more sensitivity than CEA, CA19-9 in patients with intestinal disease, also the higher level of arginase as well as CEA,CA19-9 was in the left colon.

REFERENCES

(1) Parkin, D.M.; Bray, F; Ferlay, J. and Pisani, P. Global cancer statistics, 2002.(2005). CA. Cancer J. Clin. 55: 74-108.

(2) Mohandas, K. M. (2011). Colorectal cancer in India: controversies, enigmas and primary prevention. Indian. J. Gastroenterol. 30(1):3–6.

(3) Al-Shuneigat1, J.M.; Mahgoub,S.S. and Fazlul, H. (2011). Colorectal carcinoma: nucleosomes, carcinoembryonic antigen and ca 19-9 as apoptotic markers; a comparative study. Journal of Biomedical Science. 18:50.

(4) Palmqvist, R.; Engaras, B.; Lindmark, G.; Hallmans, G.; Tavelin, B.; Nillson, O.; Hammarstr,M. S.and Hafstr, M. L. (2003). Prediagnostic levels of carcinoembryonic antigen and CA 242 in colorectal cancer: a matched case-control study. Dis. Colon. Rectum. 46:1538-1544.

(5) Japar, I.; Choi,G. and Jun,S.H.(2009). Prognostic Value of Serum Tumor Antigen CA19-9mAfter Curative Resection of Colorectal Cancer. Anticancer Rsearch. 29: 4303-4308.

(6) Mielczarek-Puta, M.; Graboń, W.; Chrzanowska, A. and Barańczyk-Kuźma, A. (2008). Arginase and arginine in diagnostics of patients with colorectal cancer and patients with colorectal cancer liver metastases.Współczesna Onkologia. 12(2): 51–55.

(7) Colombo, J.I. and Konarska, L. (1984). Arginase. In: Methods of enzymatic analysis. vol.2. Enzymes, Esterases, Glycosidases, Lyases, Weinheim.15th Ed. Bergmeyer, I,.; Bergmeyer, J.and Grass, M. (eds). Verldg Chemie. x285-94.

(8) Nissim, I.; Luhovyy, B. and Horyn, O. (2005) . The role of mitochondrially bound arginase in regulation of urea synthesis. J. Biol. Chem. 280: 17715–17724.

(9) Cederbaum, S.D.; Yu, H.; Grody, W.W.; Kern; R.M.; Yoo, P. and, R.K. (2004) Arginases I and II: do their functions overlap?. Mol. Genet. Metab. 8: S38 - S44.

(10) Zea,A.H.; Rodriguez,P.C.; AtkinsM.B.; Hernandez,C. Signoretti,S.; Zabaleta,J.; McDermott,D.; Quiceno,D.; Youmans,A.; Neill,A.O.; Mier,J. and Ochoa,A.C.(2005). Arginase-Producing Myeloid Suppressor Cells in Renal Cell Carcinoma Patients: A Mechanism of Tumor Evasion. Cancer Res. 65(8):3044-8.

(11) Graboń, W.; Mielczarek-Puta, M.; Chrzanowska, A. and Barańczyk-Kuźma, A.(2009).L-arginine as a factor increasing arginase significance in diagnosis of primary and metastatic colorectal cancer.Clin Biochem. 42(4-5):353-7.

(12) Munder, M. (2009). Review Arginase: an emerging key player in the mammalian immune systembph. B. J. of Pharmacol. 158: 638–651.

(13) Astler, V.B. and Coller, F.A.(1954). The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann. Sur. 139:846-852.

(14) Leu, S. Y. and Soo-Ray Wang,S.R.(1992). Clinical Significance of Arginase in Colorectal Cancer. Cancer. 70(4) .

(15) Park, I. J. ; Chio, G. S. and Jun, S. H. (2009). Prognostic Value of Serum Tumor Antigen CA19-9After Curative Resection of Colorectal Cancer. Anticancer Research. 29: 4303-4308

(16) SAS. (2010).Statistical Analysis System, User's Guide Statistical. Version 9.1 ed. SAS.Inc. Cary. N.C. USA.

(17) Ikemoto, M.; Ishida, A.; Tsunekawa, S.; Ozawa, K.; Kasai,Y.;Totani,M.and Usda,K.(1993). Enzyme immunoassay of Type arginase and its potential clinical application.Clin. Chem. 39(5):794-79.

(18) Rodriguez, P.C.; Hernandez, C.P; Morrow, K.;Sierra, R.; Zabaleta, J.; Wyczechowska, D.D. and Ochoa, A.C.(2010). L-arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. J. Immunol. 185(9):5198-204

(19) Sidney, M. and Morris, J.r.(2012). Arginases and Arginine Deficiency Syndromes. Curr. Opin. Clin. Nutr. Metab. Care. 15(1): 64–70.

(20) Mielczarek, M.; Chrzanowska,A.;Scibior, D.;Skwarek, A.; Ashamiss, F. Lewandowska, K. and Barańczyk-Kuźma, A.(2006). Arginase as a useful factor for the diagnosis of colorectal cancer liver metastases. Int. J. Biol. Markers. 21(1):40-4.

(21) Kocna,P.; Fric,P.; Zavoral,M. and Pelech,T.(1996). Arginase Activity Determination A Marker of Large Bowel Mucosa Proliferation.Eur. J. CHn. Chem. Clin. Biochem. 34:619-623.

(22) park,I.J.; Choia,G.S.and Jun,S.H.(2009). Prognostic Value of Serum Tumor Antigen CA19-9After Curative Resection of Colorectal Cancer. Anticancer research 29: 4303-4308.

(23) Porembska, Z.; Mielczarek, M.; Nyckowski, P. and Barańczyk-Kuźma, A.(2002). [Arginase as a marker of cancerogenesis. I. Monitoring patients after resection of colorectal cancer]. Pol. Merkur. Lekarski.13(76):284-5.

(24) konarska,L.;Kolasa,T.;Albercht,p. and Regula,A.(1993).Can arginase be a marker of large be amarker of the large bowel neoplasia?. Acta. Biochemical Polonica. 40(1).

(25) Goonetilleke, K.S. and Siriwardena, A.K. (2007). Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. Eur. J. Surg. Oncol. 33 (3): 266–70.

(26) Locker, G.; Hamilton, S. ;Harris, J.; Jessup J.; Kemeny, N.; Macdonald, J.; Somerfield. M.; Hayes, D. and Bast, R. (2006). "ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer". J. Clin. Oncol. 24 (33): 5313–27.

(27) Bufill, J.A., (1990). Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann. Intern. Med. 113(10):779-788.

(28) LI,F.Y.and LAI,M.D.(2009).Review: Colorectal cancer, one entity or three. J. Zhejiang Univ. Sci. B. 10(3):219-229.

(29) Larsson, S.C.; Wolk, A. (2006). Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int. J. Cancer. 119(11):2657-2664.

(30) Ji, B.T.; Devesa, S.S.; Chow, W.H.; Jin, F. and Gao, Y.T. (1998). Colorectal cancer incidence trends by subsite in urban Shanghai, 1972-1994. Cancer Epidemiol. Biomarkers Prev. 7(8):661-666.

(31) Wu, X.; Chen, V.W.; Martin, J.; Roffers, S.; Groves, F.D.; Correa, C.N.; Hamilton-Byrd, E. and Jemal, A. 2004. Subsite-specific colorectal cancer incidence rates and stage distributions among Asians and Pacific Islanders in the United States, 1995 to 1999. Cancer Epidemiol. Biomarkers Prev. 13(7):1215-1222.

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