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Study of AnnexinA3 and Platelet Derived Endothelial Cell Growth Factor (Thymidine Phosphorylase) in Colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is a common malignant tumor of the gastrointestinal tract, constituting 10% of new colorectal cancer cases each year. Despite major breakthroughs in the treatment of colorectal cancer, 40–50% of patients are likely to develop local or distant tumor recurrences, and survival rates are poor.

Aim of the workis to evaluate the levels of annexin A3 and thymidine phosphorylase in patients with colorectal cancer and compare these levels with control persons and to ascertain whether annexin A3 and thymidine phosphorylase are involved in the progression of the colorectal cancer and their impact on patient outcome.

Patients and methods: This study was performed at Sohag University Hospital from December 2015 to December 2016 , Seventy five patients were enrolled in the study and divided into two groups. 60 patients with colorectal cancer, 15 patients with benign colorectal disease. All were matched for age and sex, for each participant body mass index (BMI), blood pressure, Annexin A3 by Eliza kit , Thymidine phosphorylase by colorimetric method were performed.

Results: There were significant relations between Annexin A3and Thymidine phosphorylase and age.Significant relations were observed between Annexin A3and Thymidine phosphorylase and type of cancer.Strong positive correlation was observed between Annexin A3and Thymidine phosphorylase.

Conculsion :we conclude that both of Annexin A3 and Thymidine phosphorylase were positive correlated with type and grade of colorectal cancer .So using Annexin A3 and thymidine phosphorylase as positive biomarker in the progression of colorectal cancer may be beneficial.

Keywords: Annexin a3,Thymidine phosphorylase,Colorectal cancer
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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide,(1) .Most CRC arises from precursor adenomatous polyps(2). stage at detection is critically related to patient survival.localized cancers (Tumour-node-metastasis[TNM]Stages1-2) have an excellent 5-yearsurvival prognosis (93%and98%); regional stage (TNM Stage3) patients have a 5-year survival rate about 60%; only 8% of patients with the late stage (TNM Stage4) disease will survive 5 years(3) .

Ca^{2+}-dependent phospholipid-binding proteins, Annexins, are involved in cellular processes, including apoptosis, proliferation and differentiation (٤).Overexpression of Annexin A1 has been found in numerous cancer types (٥). Annexin A2 induces angiogenesis by regulating the extracellular matrix metalloproteinase inducer. Furthermore,

overexpression of Annexin A2 has been associated with the development, invasion and distant metastases of various tumors (٦).

Compared with other Annexins, few previous studies have investigated the function of Annexin A3. Annexin A3 staining was markedly decreased or absent in prostate cancer and was found to correlate inversely with primary tumour stage and Gleason grade (٧).

Yan et al, (٨)previously showed that the increased expression of Annexin A3 is a mechanism of platinum resistance in ovarian cancer. Similarly, the Annexin A3 gene is silenced by micro RNA(miRNA) which is asmall non-coding RNAmolecule(containing about 22 nucleotides)and functions via base-pairing with complementary sequences with mRNA molecules.

These mRNAmolecules are silenced by one or more of the following processes:1)cleavage of the mRNA strand into two pieces,2)destabilization of its poly (A)tail,and3) less efficient translation of the mRNA into proteins by ribosomes (٩), induced apoptosis and inhibits the growth of human gallbladder cancer cells (١٠), these contradictory studies led the present study to investigate the roles of Annexin A3 expression in colorectal cancer.

Thymidine phosphorylase (TP) catalyses the conversion of thymidine and 2-deoxyuridine to their respective bases and 2-α-deoxyribose-1-phosphate (١١). TP is also involved in endothelial cell chemotaxis in vitro and angiogenesis in vivo (١٢). TP expression in various kinds of tumors is higher than in the adjacent non neoplastic tissues, and numerous studies have consistently reported that the level of TP expression in cancer cells is closely associated with malignancy and or angiogenesis (١٣). However, incolorectal carcinomas, TP expression is an independent indicator of un favorable prognosis irrespective of angiogenetic activity (١٤)

To date, 5-fluorouracil (5-FU) constitutes the fundamental basis of chemotherapy treatment for patients with colorectal cancer. The enzyme thymidine phosphorylase (TP; E.C. 2.4.2.4) catalyzes the conversion of 5-FU to its more active nucleoside form, 5-fluoro-2'-deoxyuridine, representing one of the main pathways by which this drug exerts its cytotoxic effect (١١) .

In the cell, TP is involved in pyrimidine metabolism. Previous studies have shown that TP levels are higher in tumour compared with normal tissues in a wide range of solid tumours (١٥)TP and its catalytic product, 2-deoxy-D-ribose-1-phosphate act as angiogenic factors via induction of endothelial cell migration and tube formation (١٦).In hypoxic environments, TP may also impart resistance to apoptosis (١١). TP expression is observed in tumour epithelial cells and stromal cells, particularly in tumour-associated macrophages (TAMs) (١٧).

Patients and methods

This study was performed at Sohag University Hospital from December 2015 to December 2016 , Seventy five patients were enrolled in the study and divided into two groups. 60 patients with colorectal cancer, 15 patients with benign colorectal disease. All were matched for age and sex, for each participant body mass index (BMI), blood pressure, Annexin A3 , Thymidine phosphorylase , were performed . After approval of Ethical committee of Sohag faculty of Medicine informed written consents were obtained from all individuals included in the study. All

individuals were informed regarding the tests and their clinical meanings before the study.The control group included 15 persons from outpatient clinic.

Ethical Consideration:

After approval of Ethical committee of SohagFaculty of Medicine informed written consents will be obtained from all subjects included in the study.

Method:

Blood samples will be withdrawn from patients and control persons, and then centrifuged in centrifuge system for 10 minutes. Serum will be removed then the samples will be stored in aliquot at -20 ºC until assay.

Routine assay:

-Complete blood count(CBC).
-Kidney function test
-Liver function test.

Annexin A3 by Eliza kitCAT NO(:WH-1824)., Thymidine phosphorylase by colorimetric method:Thymidine Phosphorylase determined by chemical method using the following reagents:[KrenitskyandBushby;1979];co ntinuous Spectrophotometric Rate Determination.(EC2.4.2.4)

Statistical analysis:

Data was analyzed using SPSS computer program version 22.0.Quantitative data was expressed as means ± standard deviation, median and range. Qualitative data was expressed as number and percentage. The data were tested for normality using Kolmogrov–Smirnov test which was significant indicating the use of nonparametric tests as data wasn't normally distributed. The nonparametric Mann–Whitney test was used for comparing two quantitative variables .Kruskal–Wallis test was used for comparison between more than two quantitative variables. Chi-Square test or Fisher's Exact test were used for comparison between qualitative variables. Spearman's correlation coefficient was used for measuring correlation between the two enzyme level.

Results

The present study was conducted on 60 patients with colorectal cancer, 15 patients with benign colorectal disease and20 healthy controls .Significant difference between the three studied groups according to age, levels of AST,ALT and HB.

able (1): Socio demographic characteristics and clinical data of patients in different studied groups (N. =90)

Parameter	Malignant tumor (N=60)	Benign tumor (N=15)	Healthy control (N=15)	P-value
Age Mean± S.D. Median (Range)	35.53± 12.96 33(23 – 60)	44.2± 16.94 45 (18 -75)	55± 13.68 55 (20 – 75)	<0.001¤
Sex Males (%) Female s (%)	22 (36.7%) 38 (63.3%)	5 (33.3%) 10 (66.7%)	7 (46.7%) 8 (53.3%)	.718¤¤
Family history Negative (%) Positive (%)	41 (68.3%) 19 (31.7%)	13 (86.7%) 2 (13.3%)	--	.136¤¤
Urea mg/dl Mean± S.D. Median (Range)	20.9± 7.9 16 (13 – 36)	20.6 ± 7.5 17 (14 – 34)	23.1± 6.06 27 (14 – 34)	0.570
Uric acid mg/dl Mean± S.D. Median (Range)	2.7 ± 1.2 2 (1.4 – 5.9)	2.9 ± 0.9 2.7 (1.9 – 4.2)	2.9 ± 0.9 2.7 (1.8 – 4.2)	0.140
Creatinine Mean± S.D. Median (Range)	0.8 ± 0.2 0.8 (0.2 – 1)	0.8 ± 0.2 0.8 (0.6 –1)	0.7± 0.2 0.7 (0.5 – 1)	0.196
Alkaline phosphatase IU/l Mean± S.D. Median (Range)	89.1± 7.2 90 (70 – 99)	91.5 ± 3.7 90 (87 – 99)	86.8 ± 9.2 90 (70 – 99)	0.594
AST IU/l Mean± S.D. Median (Range)	19.9 ± 7.3 21 (0.5 – 33)	24.5 ± 7.6 27 (10 – 33)	22.9 ± 4.9 23 (20 – 40)	0.017¤
ALT IU/l Mean± S.D. Median (Range)	16.6 ± 4.5 16 (6 – 23)	23.4 ± 5.2 23 (16 – 34)	19.3 ± 7.4 18 (13 – 45)	0.000¤
HB g/dl Mean± S.D. Median (Range)	10.02 ± 2.04 9.8 (5.5 – 13)	10.3 ± 2.4 9.9 (7.6 – 15.1)	12.5 ± 2.01 13 (7.7 – 15)	0.000¤
RBCs×10^6ul Mean± S.D. Median (Range)	4.2 ± 0.8 4.1 (2.6 – 5.5)	3.9 ± 1.6 4.6 (1.2 – 5.6)	4.9 ± 0.5 4.9 (3.6 – 5.5)	0.052
WBCs×10^3/ul Mean± S.D. Median (Range)	10.02 ± 4.2 9.2 (5.5 – 17.6)	8.7 ± 2.5 8 (5.5 – 14.2)	8.9 ± 2.3 8 (5.8 – 14.6)	0.789
Platelets ×10^3/ul Mean± S.D. Median (Range)	354.5 ± 121.5 345 (219 – 617)	306.3 ± 62.1 299 (220 – 400)	291.1 ± 81.4 250 (220 – 486)	0.108

AST:Aspartate transaminase ALT:Alanine transaminase
HB:HaemoglobinRBCs:Red Blood Cell count WBCs: White Blood Cell count
¤P- value was calculated by Kruskal Wallis test ¤¤P- value was calculated Chi square test

Table (2): Comparison of Thymidine phosphorylase and Annexin A3 levels among the study groups

Thymidine phosphorylase Units/ml	Healthy control (N= 60)	Benign tumor (N= 15)	Malignant tumor (N= 15)	P-value	P1	P2	P3
Mean± S.D. Median (Range)	29.09± 5.73 27.3 (19.3 – 35.6)	70.85± 4.59 70.6 (65.1 -77.1)	147.64± 13.33 147 (123.2 – 171.3)	<0.001*	<0.001*	<0.001*	<0.001*
Annexin A3 Pg/ml							
Mean± S.D. Median (Range)	67.68± 14.36 70.2 (34.4 – 80.9)	162.31± 20.33 165 (134.9 -198)	997.68± 542.62 1100 (200 – 1780)	<0.001*	<0.001*	<0.001*	<0.001*

P- value was calculated by Kruskal Wallis test among three groups
P1 by Mann–Whitney test, between the benign group and the malignant group
P2 by Mann–Whitney test, between the benign group and healthy group
P3 by Mann–Whitney test, between malignant group and healthy group
*Statistically significant
Significant difference between Annexin A3 and Thymidine phosphorylase according to type of colorectal cancer ,Table2.

Table (3): Comparison of Thymidine phosphorylase and Annexin A3 according to tumor burden (N. = 60)

Enzyme	High (N= 23)	Low (N= 37)	P-value
Thymidine phosphorylase (Units/ml) Mean± S.D. Median (Range)	153.42± 12.59 152.6 (130.2 – 171.3)	144.05 ± 12.64 146.5 (123.2 – 169.9)	0.022*
Annexin A3(Pg/ml) Mean± S.D. Median (Range)	1429.65 ± 326.32 1500 (599 – 1780)	729.16± 473.51 500 (200 – 1700)	0.000*

P- value was calculated by Mann–Whitney test *Statistically significant
Significant difference between Annexin A3 and Thymidine phosphorylase according to tumour burden Table3.

Table (4): Comparison of Thymidine phosphorylase level and Annexin A3 according to tumor grading (N. = 60)

Thymidine phosphorylase	**Grade 2 (N= 22)**	**Grade 3 (N= 18)**	**Grade 4 (N=20)**	**P-value**
Mean± S.D. Median (Range)	141.9± 11.4 146.5 (123.2 – 163.4)	146.9± 13.9 147.3 (126.8 -169.9)	154.5± 12.1 154.3 (136.8 – 171.3)	0.035*
Annexin A3				
Mean± S.D. Median (Range)	342.6± 125.5 330 (200 – 600)	1171± 124.6 1150 (1000 - 1500)	1562.3± 149.3 1585 (1334 - 1780)	<0.001*

P- value was calculated by Kruskal Wallis test *Statistically significant
Significant difference between Annexin A3 and Thymidine phosphorylase according to tumour grading ,Table4.

Table (5): Correlation between Thymidine phosphorylase and Annexin A3 levels in all the studied groups (n=90).

Enzyme	Annexina3	
	r	P-value
Thymidine phosphorylase	0.791	<0.001*

r = Spearman's correlation coefficient. *Statistically significant correlation

Strong positive significant relation between Annexin A3 and Thymidine phosphorylase in all the studied groups,Table5.

Table (6): Correlation between Thymidine phosphorylase and Annexin A3 levels among the three studied group (n= 90).

Group	r	P-value
Malignant tumor group	0.311	0.016*
Benign tumor group	0.320	0.245
Control group	0.513	0.051

r = Spearman's correlation coefficient. *Statistically significant correlation

Significant correlation between Annexin A3 and Thymidine phosphorylase among malignant tumourgroup,Table 6.

Discussion

Colorectal cancer (CRC) is a disease with a major world -wide burden. It is the fourth most frequently diagnosed malignancy in men and third most common in women, with almost one million people developing CRC annually(١٨).In the world, CRC is the third most common cause of cancer death, responsible for 639,000 deaths annually(١٩).

Annexin family proteins are a well-known multi gene family of Ca2 -regulated phospholipid- and membrane-binding proteins. The summarized research evidences in recent years indicate Anxa3 might specifically functionalize either as a tumor suppressor or as a tumor promoter candidate for different cancer depending on the types of tumor cells and tissues.(٢٠).

The association of Anxa3 with colorectal cancer

Anx a3 in serum:

Anxa3 might be utilized as one potential biomarker in the diagnosis, treatment and prognos is of CRC. Yip et al,(1٩) examined the levels of seven potential gene biomarkers in the blood samples collected from Malaysia and North American patients with CRC by quantitative RT-PCR(qRT-PCR) analysis(٢١) . Anxa3 was significantly up-regulated in CRC patients. Comparing to blood samples from healthy people, the averaged Anxa3 levels in patients specimens from North American and Malaysian were increased by 171 and 206 %, respectively, with statistical significance (P \0.0001).

This agreed with our result which showed significant difference between Anxa3 in the three studied groups (mean in malignant group 997.68±542.62, in benign group 162.31±20.33,in healthy control 67.68±14.36)(p value <0.001).

This reported that Annexin A3 has been associated with the stimulation of of vascular endothelial growth factor (VEGF) expression and may be a regulator of angiogenesis. Park et al,2005 found that Annexin A3-overexpressing

human embryonic kidney (HEK) 293 cells induce the migration and tube formation of human umbilical vein endothelial cells. Furthermore, in HEK 293 cells, the expression of Annexin A3 activates Hypoxia-inducible factor-1α(HIF-1α)transactivation activity, which indicates that Annexin A3 may regulate the HIF-1 signaling pathway. Therefore, Annexin A3 has been hypothesized to be vital in the angiogenesis of cancer.

Anx A3 in tissues:

Correlation between Annexin A3 expression and clinic pathological variables. Of the 60 colorectal cancer tissues, Annexin A3 staining was undetected in 21 cases. Compared with weak or no expression in normal colorectal tissues, 65% (39/60) of colorectal cancer specimens showed Annexin A3 immunoreactivity . Annexin A3 was predominantly expressed in the cytoplasm of cancer cells. To improve the investigation of the role of Annexin A3 in colorectal cancer, the correlation between Annexin A3 expression and clinicopathological factors was analyzed. Tumor size and Dukes' stage exhibited statistically significant correlations with Annexin A3 expression . However, no significant correlation was identified between the levels of Annexin A3 expression and other clinical and pathological features, including gender, age, tumor localization, tumor differentiation degree and lymph node metastasis(٢٢).

The association of thymidine phosphorylase with colorectal cancer:

Thymidine phosphorylase (TP) is well-known to be identical with platelet-derived endothelial cell growth factor (PD-ECGF) as an angiogenic factor. In addition, TP is also known to be an essential enzyme for the activation of capecitabine, a new oral fluoropyrimidine, which is now being globally prescribed for colorectal and breast cancer patients as a standard chemotherapy regimen (٢٣).

Thymidine phosphorylase in serum:

From 88 patients with colorectal cancer, specimens of venous blood drainage were obtained during operation. The serum TP levels were measured by ahighly sensitive Enzyme Linked Immunosorbent Assay(ELIZA)method. Subsequently,88 patients were divided into two groups based on the levels ofTP. The dividing line was determined to be55ng/ml.The TP-high group (greater than;55ng/ml)had asignificantly shorter overall survival than the TP-lowgroup(lower than;55ng/ml). Amultivariate analysis indicated that the serumTP level in venous blood drainage specimens to be abetter prognostic factor independent of the traditional pathologic parameters (٢٤).

This agreed with our result which showed significant difference between TP in the three studied groups (mean in malignant group 147.64±13.33 ,in benign group 70.85±4.59,in healthy control 29.09±5.73)(p value <0.001).

This reported that TP and its catalytic product, 2-deoxy-D-ribose-1-phosphate act as angiogenicfactors via induction of endothelial cell migration and tube formation (٢٥).

In hypoxic environments,TP may also impart resistance to apoptosis (11,٢٦). TP expression is observed in tumour epithelial cells and stromal cells, particularly in tumour-associated macrophages(TAMs) (16,17,٢٧).

We conclude that both of Annexin A3 and Thymidine phosphorylase were positive correlated with type and grade of colorectal cancer .So using Annexin A3 and Thymidine phosphorylase as

positive biomarker in the progression of colorectal cancer may be beneficial.

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