The Egyptian Journal of Biochemistry & Molecular Biology
VOL 33 (N.1،2) 16-33 December. 2015

A PRELIMINARY STUDY ON ENDOTHELIAL NITRIC OXIDE SYNTHASE INTRON 4a/b POLYMORPHISM IN ULCERATIVE COLITIS-ASSOCIATED COLORECTAL CANCER

HANAA HIBISHY GABALLAH[1]*,SABER ABDELRAHMAN ISMAIL[2]

[1]MEDICAL BIOCHEMISTRY DEPARTMENT, [2]TROPICAL MEDICINE DEPARTMENT, FACULTY OF MEDICINE, TANTA UNIVERSITY, EGYPT

Received 16/2/2015– Accepted 30/3/2015

ABSTRACT

Cancer is one the most concerning complications of longstanding ulcerative colitis (UC). This study was undertaken to investigate the possible association between eNOS gene intron 4 polymorphism and the development of UC-associated colorectal cancer. It was carried out on 120 subjects; distributed as follows: 40 patients with UC associated colorectal cancer, 40 patients with UC who did not develop colorectal cancer and 40 control subjects. Genotypes (aa, bb, ab) for eNOS gene intron 4 polymorphism were identified using amplified fragment length polymorphism PCR. Plasma nitrate and nitrite levels were used to estimate the amounts of endogenous nitric oxide formation using nitric oxide colorimetric assay kit. Our preliminary data revealed that, compared to the bb genotype, the aa and ab eNOS genotypes were significantly associated with increased risk of developing UC associated colorectal cancer. Meanwhile, in UC associated - colorectal cancer and UC patients groups, plasma NO levels were higher in patients with 4a4a/4a4b genotypes compared to those with bb genotype. It can be concluded from our preliminary

* *Correspondence Author (e mail: (hanaahibishy @hotmail.com)*

study that a allele variant of eNOS intron 4 polymorphism may be associated with increased risk of development of UC associated colorectal cancer, however, large scaled studies are needed to verify these preliminary results.

Key words: Endothelial Nitric Oxide Synthase; ulcerative colitis; colorectal cancer; intron 4 polymorphism.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disorder of the intestinal tract. Patients with UC frequently experience episodes of bloody diarrhea with or without mucous, abdominal pain, fever and weight loss **(Tontini et al., 2015).** Several factors are associated with the initiation and enhancement of chronic inflammatory response in UC including immunologic abnormalities, genetic influences, environmental agents, altered colonic barrier function, bacterial and viral infections as well as nutrition and psychosocial factors **(Leone et al., 2013).** The development of dysplasia and cancer is one the most concerning complications of longstanding UC **(Parian and Lazarev 2015).** Thus, noninvasive screening tools that can identify UC patients at high risk for developing colorectal cancer are urgently required.

Nitric oxide (NO) is a short-lived, pleiotropic free radical molecule produced by nitric oxide synthase (NOS) enzyme; in a reaction that converts arginine and oxygen into citrulline and NO **(Toledo and Augusto 2012).** Three isoforms of NOS exist, depending on the site of origin: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) **(Förstermann and Sessa 2012).** NO plays a double edged role in modulating the intestinal mucosal integrity; as it can exert both protective and proinflammatory actions. NO, formed physiologically by constitutive NO synthases, regulates vascular tone, protects the microvasculature from injury, and modulates adhesion of inflammatory cells. In contrast, NO has also been associated with the

initiation and maintenance of inflammation in human inflammatory bowel disease **(Lundberg and Weitzberg 2013).**

The gene encoding eNOS maps to chromosome 7q35-7q36; contains 26 exons with a total size of 21 kb and encodes for a protein of 1203 amino acids **(Shankarishan et al., 2014).** The eNOS 4a/b polymorphism of the 27-bp variable number tandem repeats (VNTR) in intron 4 has two alleles: a common large allele and a smaller allele. The larger allele (eNOS4b allele), designated 'b-insertion' has five tandem repeats, and the smaller allele (eNOS4a allele) 'a-deletion' has four repeats **(Ekmekçi et al., 2013).** This polymorphism been associated with changes in plasma NO levels **(Zhang et al., 2008 a).** Several epidemiological studies have investigated the role of eNOS polymorphisms in different types of human cancer including vulvar, prostate, endometrial and breast cancer **(Zhang et al., 2014).** However; the role of eNOS 4a/b polymorphism in the development of UC-associated colorectal cancer remains to be elucidated. Therefore, we aimed to investigate the possible association between eNOS gene intron 4 polymorphism and the development of UC associated colorectal cancer in order to assess its role as a discriminatory genetic marker for identification of UC patients at high risk for developing cancer.

MATERIALS AND METHODS

This study included eighty ulcerative colitis patients selected from the inpatients and outpatients clinics of the Tropical Medicine Department of Tanta University Hospital, Egypt .They were divided into: forty ulcerative colitis patients with colorectal cancer (18male, 22 female), aged (52.62±7.74) years; besides forty patients with ulcerative colitis without colorectal cancer (15 male, 25 female), aged (49.59± 5.80) years. The diagnosis of UC was based upon a clinical history of

diarrhea and/or rectal bleeding for 6 wk or more, typical radiological and endoscopic findings, and characteristic microscopic changes on biopsy specimens. Disease duration was calculated from the date of diagnosis of UC, taken from the medical records. In addition, forty age and sex-matched subjects, who visited other departments of the hospital seeking treatment for non-neoplastic diseases and who have no family history of cancer were recruited for participation as controls. All subjects gave their written informed consent before participation. The study protocol was approved by the local ethics committee at Faculty of medicine; Tanta University, and was in accordance with the principles of the Declaration of Helsinki II.

Inclusion criteria for cases: UC- colorectal cancer patients group included patients who had colorectal cancer on top of UC and who had UC disease duration not less than than 10 years (to exclude sporadic colorectal cancer). The lesions were histologically confirmed by a pathologist according to the World Health Organization histological typing. For UC-no colorectal cancer patients: only patients with histologically confirmed UC who did not develop colorectal cancer or any grade of dysplasia and who underwent colonoscopy with biopsy were selected. Group I and II patients were frequency matched on duration and extent of colitis, age, ethnicity, and gender.

Exclusion criteria: Patients with other confounding pathologies in the colon, autoimmune disease, heart failure, renal failure, chronic liver disease, thyroid disorders, acute infection, stroke and patients with prior radiotherapy, immunosuppressive or chemotherapeutic drugs were excluded from the study.

All patients and control were subjected to complete history taking and thorough clinical examination.

Colonic biopsy samples: Ulcerative colitis cases underwent colonic biopsy sampling during surveillance colonoscopy, which was

performed according to established American Gastroenterological Association guidelines for UC surveillance colonoscopy **(Winawer et al., 2003).** UC- colorectal cancer cases underwent surgical resection .Colonic biopsy specimens obtained at endoscopy or by colectomy were immediately fixed in formaldehyde for the histopathological examination.

Blood sampling: After 12 hours of overnight fasting, 5ml of venous blood samples were taken from each studied subject and kept in EDTA 5% coated tubes. 2.5 ml EDTA treated- blood was separated and stored at –20°C until DNA isolation. Plasma was separated after centrifugation of the other set of EDTA-treated tubes for the NO assay.

Routine laboratory investigations were performed for all patients and controls including, complete blood picture, blood urea and serum creatinine, total lipid profile, erythrocyte sedimentation rate and stool examination to exclude bacterial causes of colitis.

DNA extraction: Genomic DNA was extracted from the whole blood with EDTA using the GF-1 total DNA Extraction Kit supplied by (Vivantis Inc., CA, USA). DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm. The extracted DNA was stored at -20 °C until analysis.

Genotyping for the endothelial nitric oxide synthase intron 4a/b Polymorphism: Polymerase Chain Reaction (PCR) was performed to determine the different genotypes of Endothelial Nitric Oxide Synthase Intron 4a/b Polymorphism. The oligonucleotide primer sequences used were designed according to **Ekmekçi et al., 2013** as follows: 5′-AGG CCC TAT GGT AGT GCC TTT-3′ (forward) and 5′-TCT CTT TAG TGC TGT GGT CAC-3′ (reverse). Briefly, the protocol consisted of 35 cycles of DNA denaturation at 95°C for 1 min, primer annealing at 60°C for 45 s and chain extension at 72°C for 1 min, followed by a final extension cycle at 72°C for 5 min. The

constituents of the reaction consisted of: 1.2 µM of each primer, 10 mM of dNTPs, 2 mM of MgCl2, 1 U of Taq DNA polymerase enzyme and 1× PCR buffer, along with 40–50 ng of DNA. The PCR products were separated in a 2% agarose electrophoresis system and the bands were visualized with ethidium bromide staining under ultraviolet (UV) trans-illumination. Two alleles were obtained when this region was amplified: "eNOS4a," which was 393 bp long and consisted of four 27-bp repeating units, and "eNOS4b," which was 420 bp long and consisted of five 27-bp repeating unit.

Measurement of plasma total nitrate/nitrite: NO production was determined in a two-step process using nitric oxide colorimetric assay kit (#CAT K262-200, BioVision, CA,USA). In the first step, nitrate was converted to nitrite using nitrate reductase. In the second step, Griess reagents were used to convert nitrite to a deep purple azo compound. The amount of the azo chromophore accurately reflected the amount of NO in the samples. Briefly, 50 µl samples of plasma were mixed with equal volumes of 1% sulfanilamide and 1 mg/ml solution of N-1-naphthyl-ethylenediamine dihydrochloride in 0.5% H3PO4. After 10 min, the absorbance was measured at 540 nm **(Giustarini et al., 2008).**

Statistical analysis: The data were analyzed using statistical package for the social science (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA). Frequencies of genotypes/alleles were determined by the gene counting method. Accordance with the Hardy-Weinberg's equilibrium, which indicates an absence of discrepancy between genotype and allele frequencies, was checked using a chi-square goodness-of-fit test. Chi-square tests were used for categorical data and Student's t-tests for continuous data. Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. the significance level of all tests was set at $P<0.05$.

RESULTS

The demographic and clinical characteristics of study groups are shown in Table 1. The differences in distributions of age, sex, BMI, blood pressure and smoking status between the patients and controls were not statistically significant (P >0.05). Three genotypes for eNOS4 a/b polymorphism were recognized by genotyping; homozygous eNOS b/b genotype with one band at 420 bp, homozygous a/a genotype with one band at 393 bp and heterozygous a/b genotype with 2 bands at 420-bp and 393-bp figure (1).

The distribution of genotypes of eNOS4 a/b polymorphism in controls were consistent with expectations under the Hardy–Weinberg equilibrium (X^2=0.21, p=0.64). The genotypic and allelic frequencies of the intron 4a/b eNOS gene polymorphisms in patients and controls are demonstrated in Table 2. The b/b genotype and the b allele were taken as references. The frequencies of the a/a and a/b eNOS genotypes were significantly higher in UC- CRC patients (20 and 37.5 % respectively) than UC-no CRC patients (5 and 22.5 % respectively) , where the odds ratios for the (a/a, and a/b) genotypes were 6.82 (95% CI : 1.29 -35.92) and 2.84 (95% CI : 1.02- 7.88) respectively, p < 0.05. Meanwhile, b/b genotype frequency was significantly lower in UC- CRC patients than UC-no CRC patients (42.5% vs 72.5. %, p < 0.05). Likewise, the frequency of 4a allele was significantly higher in UC- CRC patients (38.75%) than UC-no CRC patients (16.25%), where its odds ratio was 3.26 (95% CI : 1.54-6.86) , p < 0.05 , lending support to the hypothesis that 4a allele might confer an increased risk for the development of UC associated cancer.

Furthermore, the a/a and a/b genotypes were significantly higher in UC- CRC patients (20 and 37.5 % respectively) compared to control subjects (2.5 and 20 % respectively), where their odds ratios were 7.29 (95% CI: 1.38 - 38.30) and 3.41 (95% CI: 1.2- 9.6), respectively, p <

0.05. Also, the 4a allele frequency was significantly higher in UC-CRC patients (38.75%) than control subjects (12.5%), where its odds ratio was 4.42 (95% CI: 1.98-9.86), $p < 0.05$. On the other hand, the distribution of the intron 4a/b eNOS genotypes did not differ significantly between UC patients and controls. Plasma NO levels were significantly higher in UC-CRC patients (88.87±30.33 µ mol/L) compared to both UC-no CRC patients and controls (70.26±22.96, 51.66±16.03µ mol/L respectively), $p<0.05$. Also, Hemoglobin levels and erythrocyte sedimentation rates showed statistically significant difference between the patients and control groups. On the other hand, fasting blood glucose, TAG, HDL-C, LDL-C and TC levels as well as total lecuocytic and platelet counts did not differ significantly between patients and control groups, $p>0.05$; these data are summarized in table 3.

To further assess the association of different 4a/b eNOS genotypes with plasma NO levels, both patients and control groups were subdivided according to 4a/b genotype, where higher plasma NO levels were significantly associated with the minor 4a allele carriers (4a4b and 4a4a genotypes) compared to 4b4b genotype. Meanwhile, in UC-CRC and UC-no CRC patients groups, plasma NO levels were higher in patients with 4a4a/4a4b genotypes (105.6±25.47, 82.6±24.24 µ mol/L respectively) as compared to those with bb genotype (66.23 ±20.11, 64.31 ±20.16 µ mol/L respectively), $p<0.05$.These data are illustrated in table 4. In conjunction with these findings, table ° showed that within the UC-CRC group; a allele variant was significantly associated with tumor stage and lymph node involvement.

Table 1: Demographic and clinical characteristics of patients and controls.

Variables	Controls (n=40)	UC- CRC Patients (n=40)	UC-no CRC Patients (n=40)	Statistical test F/X^2
Age	51.6± 7.0	52.6±7.7	49.5± 5.8	F=1.9
Gender, N (%)				
Female	18(45%)	22(55%)	25(62.5%)	X2= 2.49
Male	22(55%)	18(45%)	15(37.5%)	
BMI (Kg/m2)	24.5±3.1	24.3± 2.3	23.5± 2.7	F=1.30
Systolic blood pressure	127.3±5.7	130.5± 17.1	125.8± 14.6	F=1.27
Diastolic blood pressure	75.1± 10.3	76.1± 5.8	77.4±7.0	F=0.81
Mean duration of UC, years	-	21.5±11.8	22.6±10.5	-
Smoking status (%)				
yes	11	10	9	X^2= 0.26
NO	29	30	31	

Data presented as means± SD or percentage. *P was considered significant at <0.05.

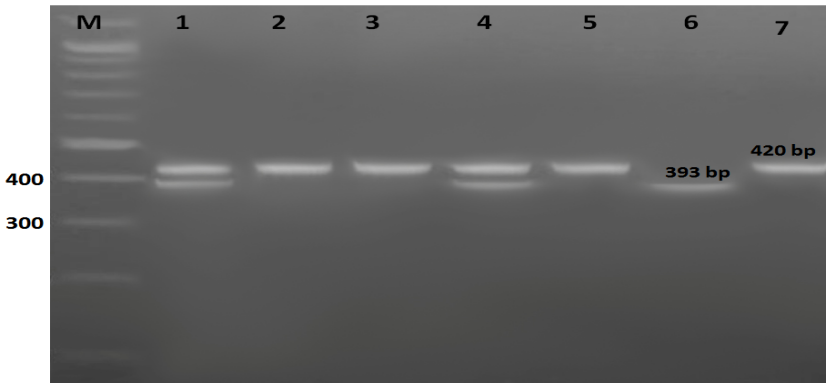

Figure 1. Gel Electrophoresis demonstrating the genotypes of eNOS intron 4 a/b polymorphism after PCR analysis. Lane M shows DNA marker (100bp), Lanes (1,4) show heterozygous a/b genotype with 2 bands at 420-bp and 393-bp, Lanes (2,3,5,7) show homozygous b/b genotype with one band at 420 bp, Lane 6 shows homozygous a/a genotype with one band at 393 bp.

Table 2: Genotypic and allelic frequencies of the Intron 4a/b eNOS gene polymorphisms in patients and controls.

eNOS Geno typing	Controls	UC- CRC	UC-no CRC	UC- CRC Vs UC-no CRC Patients	UC- CRC Patients Vs Controls	UC-no CRC Patients Vs Controls
	Number (frequency)	Number (frequency)	Number (frequency)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
4b4b	31(0.775)	17(0.425)	29(0.725)	1.00 Reference	1.00 Reference	1.00 Reference
4a4b	8(0.2)	15(0.375)	9(0.225)	2.84 (1.02- 7.8) *	3.41 (1.2- 9.6) *	1.20 (0.4- 3.5)
4a4a	1(0.025)	8(0.2)	2(0.05)	6.82 (1.2 -35.9) *	7.29 (1.3 -38.3) *	2.1 (0.1-24.8)
4b allele	70(0.875)	49(0.6125)	67(0.8375)	1.00 Reference	1.00 Reference	1.00 Reference
4a allele	10(0.125)	31(0.3875)	13(0.1625)	3.26 (1.5-6.8) *	4.42 (1.9-9.8) *	1.3 (0.5-3.3)

CI: confidence interval, * P was considered significant at <0.05.

Table 3: Biochemical parameters of the studied groups:

Parameters	Controls	UC- CRC	UC-no CRC	ANOVA (F value)
Plasma nitric oxide (µ mol/L)	51.6±16.0[a,b]	88.8±30.3	70.2±22.9[a]	24.3*
Fasting blood glucose (g/dl)	89.2±7.35	88.6±8.0	91.4±2.6	2.1
HDL-C (mg/dl)	50.4±9.2	48.1±9.8	48.8±5.05	0.8
TAG (mg/dl)	92.5±15.5	96.6±13.5	93.3±10.7	1.1
TC (mg/dl)	165.7±25.7	175.5±11.4	171.1±14.7	2.8
LDL-C (mg/dl)	127.7±18.2	132.7±9.3	128.1±13.2	1.5
ESR (mm/hr)	10.28±2.45[a,b]	42.6±10.3	21.7±6.3[a]	209.7*
Hemoglobi (gm/dl)	12.3±1.6[a,b]	10.9±0.9	11.9±0.6[a]	16.4*
Platelet count (104/mm3)	35.3±4.67	34.3±5.1	35.8±5.7	0.9
White blood cells /mm3	5136.5±851.8	5270.7±890.1	5429.9±1094.2	0.9

Data are represented as mean ± SD; * significant at p<0.05; a: significant when compared to UC-CRC group, b: significant when compared to UC-no CRC group.

Table 4: Plasma NO levels in various genotypes of Intron 4a/b eNOS Polymorphism

Groups	Plasma NO levels (µ mol/L) in different Intron 4a/b eNOS genotypes	
	4b4b	4a4a /4a4b
Controls	49.01 ± 16.69	59.62 ± 11.04*
UC- CRC	66.23 ± 20.11	105.6 ± 25.47*
UC-no CRC	64.31 ± 20.16	82.6 ± 24.24*

Data presented as means± SD, P was considered significant at <0.05,
*: significant when compared to 4b4b.

Table (5): Association of Intron 4a/b eNOS genotypes with clinicopathological features in UC-colorectal cancer patients.

Clinicopathological features		4b4b		aa /ab		Total	
		N	%	N	%	N	%
Gender	Female	10	58.82	12	52.17	22	55
	Male	7	41.18	11	47.83	18	45
Tumor site	Colon	12	70.59	16	69.57	28	70
	Rectum	5	29.41	7	30.43	12	30
TNM Tumor stage	T1+T2	10	58.82	6	26.08*	16	40
	T3+T4	7	41.18	17	73.91*	24	60
lymph node involvement	Positive	6	35.29	16	69.56*	17	42.5
	Negative	11	64.71	7	30.44*	23	57.5

P was calculated by Chi-square test. P was considered significant at <0.05, *: significant when compared to 4b4b

DISCUSSION

Our preliminary data pointed out that the (a) variant genotypes of the 4a/4b polymorphism (4b4a+4a4a) were significantly associated with an increased risk of UC -colorectal cancer with reference to the bb genotype, indicating a potentially crucial role for this gene in the development of UC-colorectal cancer. These data are consistent with the findings of **Yeh et al. 2009** who suggested that the eNOS 4a allele was associated with a predisposition to early-onset colorectal cancer among a Chinese population in Taiwan. They concluded that the (4b4a+4a4a) eNOS genotypes were associated with a 69% increase in colorectal cancer risk among subjects less than 60 years old, compared with the bb genotype. However, the study by **Jang et al. 2013** did not support such an association between the 4a4b polymorphism and colorectal cancer risk in the Korean population. Well in line, several studies have reported that the eNOS 4a allele was positively associated with cancer susceptibility in breast **(Ramírez-Patiño et al., 2013)**, prostate **(Safarinejad et al., 2013),** and vulva **(Riener et al., 2004)** cancer patients. However, other studies have shown no correlation between the eNOS 4a4b polymorphism and cancer risk

(Zhang et al., 2014). These contradictions could be partly attributed to differences in disease state, sample size and ethnicity as well as intricacy of gene expression regulation or divergent biological pathways controlling disease pathogenesis **(Jang et al., 2013)**. Furthermore, we sought to measure the plasma nitric oxide levels and to examine their correlations with different eNOS 4a/4b genotypes in the patients and controls. Our data revealed that plasma NO levels were significantly increased in patients with UC-colorectal cancer compared to those with UC-no colorectal cancer as well as the control group. This finding is in accord with that of **Yagihashi et al., 2000** who showed that the expression of eNOS in human colorectal cancers was enhanced and correlated with tumor growth and vascular invasion. According to **Mortensen et al., 2004**, colorectal peritumoral microvessels were found to express high levels of immunoreactive eNOS. Meanwhile, overexpression of eNOS was identified as an early event in rat colon tumor development, occurring even before morphological changes associated with tumor generation were observed **(Escribano et al., 2004).** Moreover, our data revealed that the NO levels were significantly increased in the carriers of the eNOS 4a allele than in non carriers. Despite being intronic, the eNOS intron 4 a/b polymorphism is reported to modulate transcription, influencing translation efficiency, mRNA stability, and enzyme levels **(Shankarishan et al., 2014).**

Concurring with this finding, functional studies have shown that the variant a allele is strongly correlated with higher plasma levels of NO and its metabolites **(Zhang et al., 2008 a)**. The eNOS intron 4 a/b polymorphism has three alleles: a short 4 repeat allele (a-allele), a more common long 5 repeat allele (b-allele), and a rare allele with 6 repeats **(Ekmekçi et al.,2013).** These repeats are the source of 27 bp short intronic repeat small RNA (sir-RNA), derived from pre-mRNA splicing, which could either be a new class of small RNA or an atypical form of the microRNA that functions as a negative feedback self regulator by specifically inhibiting the eNOS gene expression at the transcriptional level. **(Zhang et al.,2008 -b)**. Conceivably, **Zhang**

et al. (2008) have demonstrated that the amount of this sir-RNA is determined by the number of repeats in intron 4,thus cells containing five 27-bp repeats (b-allele) produced higher levels of this sir-RNA and lower levels of eNOS mRNA than cells with four 27-bp repeats (a-allele).

Further bolstering these findings, our data reported a significant association between a allele variant and the clinicopathological features of colorectal cancer such as advanced tumor stage and lymph nodes involvement. Taken together, it is tempting to speculate that the individual genetic profile with the a-allele genotypes (aa and ab) of the ecNOS4 a/b polymorphism might contribute to the increased susceptibility to UC- associated colorectal cancer by increasing NO production. This finding is biologically plausible as prevailing experimental and clinical data suggest a promoting role of NO in tumor growth, progression and metastasis in some solid tumors, including pancreatic, breast and ovarian cancer **(Burke et al., 2013).** In this context, it was reported that elevated NO can cause mutagenesis through several mechanisms including DNA damage, transition and or transversion of nucleic acid bases and inactivation of DNA-repair proteins **(Ohnishi et al.,2013).** NO may also enhance cancer progression by activating several oncogenic signaling pathways (such as extracellular signal-regulated kinases and phosphoinositide 3-kinases) in addition to inactivating tumor suppressor oncoprotein P53 **(Ramírez-Patiño et al., 2013).** Moreover, **Lim et al., 2008** reported that blocking phosphorylation of eNOS inhibited tumor initiation and maintenance through enhancement of the nitrosylation and activation of endogenous wild-type Ras proteins, which are required throughout tumorigenesis. Meanwhile, NO upregulates matrix metalloproteases and promotes VEGF-induced angiogenesis, tumor vascularization and invasion **(Zhuang et al., 2013).**

On the other hand, NO derived from eNOS was found to be inversely correlated with promotion, progression and metastatic capability of other malignant solid tumors such as non-small-cell lung cancer

(Fujita et al., 2010). Given that the role of NO in cancer is multidimensional; depending on the activity and localization of NOS isoforms, concentration and duration of NO exposure and cellular sensitivity, it is conceivable that the differential sensitivity of tumor cells to NO-mediated cytostasis or apoptosis and clonal evolution of NO-resistant and NO-dependent cells could rationalize the dual and contradictory role of NO in carcinogenesis **(Burke et al., 2013).**

Our study has some limitations that worth mentioning. First, the sample size is relatively small; therefore, our findings should be interpreted with caution and verified using larger scaled studies. Also, because this study is restricted to the Egyptian population; these results may not be applied to the patients of other ethnic or racial groups.

In conclusion, our preliminary study demonstrated that the a allele was overrepresented in UC associated colorectal cancer patients and was associated with higher NO plasma levels, suggesting that this allelic variation of eNOS may play a role as a risk factor in the pathogenesis of UC associated colorectal cancer development . Our s preliminary data suggested that the 4a4b ecNOS gene polymorphism could serve as a useful genetic marker for evaluation of susceptibility to UC associated colorectal cancer as well as a potential therapeutic gene target for UC patients.

REFERENCES

Burke AJ, Sullivan FJ, Giles FJ, Glynn SA. (2013):The yin and yang of nitric oxide in cancer progression.Carcinogenesis34(3):503-12

Ekmekçi A, Ozcan KS, Güngör B, Abaci N, et al. (2013): The relationship between endothelial nitric oxide synthase 4a/4b gene polymorphism and premature coronary artery disease. Acta Cardiol. 68(5):464-8.

Escribano M, Molero L, López-Farré A, Abarrategui C, et al. (2004): Aspirin inhibits endothelial nitric oxide synthase (eNOS) and Flk-1 (vascular endothelial growth factor receptor-2) prior to rat colon tumour development. Clin Sci (Lond). 106(1):83-91.

Förstermann U, Sessa WC. (2012): Nitric oxide synthases: regulation and function. Eur Heart J. 33(7):829-37, 837a-837d.

Fujita S, Masago K, Hatachi Y, Fukuhara A, et al. (2010): Genetic polymorphisms in the endothelial nitric oxide synthase gene correlate with overall survival in advanced non-small lung cancer patients treated with platinum-based doublet chemotherapy. BMC Med Genet; 11:167.

Giustarini D, Rossi R, Milzani A, Dalle-Donne I. (2008): Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization. Methods Enzymol. 440:361-80.

Jang MJ, Jeon YJ, Kim JW, Chong SY, et al. (2013): Association of eNOS polymorphisms (-786T>C, 4a4b, 894G>T) with colorectal cancer susceptibility in the Korean population. Gene; 512(2):275-81.

Leone V, Chang EB, Devkota S. (2013): Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. J Gastroenterol. 48(3):315-21.

Lim KH, Ancrile BB, Kashatus DF, Counter CM (2008): Tumour maintenance is mediated by eNOS. Nature, 452:646-649.

Lundberg JO, Weitzberg E. (2013): Biology of nitrogen oxides in the gastrointestinal tract. Gut. 62(4):616-29.

Mortensen K, Christensen IJ, Nielsen HJ, Hansen U, et al. (2004): High expression of endothelial cell nitric oxide synthase in peritumoral microvessels predicts increased disease-free survival in colorectal cancer. Cancer Lett. 216(1):109-14.

Ohnishi S, Ma N, Thanan R, Pinlaor S, et al. (2013): DNA damage in inflammation-related carcinogenesis and cancer stem cells. Oxid Med Cell Longev. 2013:387014.

Parian A, Lazarev M. (2015): Who and how to screen for cancer in at-risk inflammatory bowel disease patients. Expert Rev Gastroenterol Hepatol.:1-16.

Ramírez-Patiño R, Figuera LE, Puebla-Pérez AM, Delgado-Saucedo JI, et al. (2013): Intron 4 VNTR (4a/b) polymorphism of the endothelial nitric oxide synthase gene is associated with breast cancer in Mexican women. J Korean Med Sci. 28(11):1587-94.

Riener EK, Hefler LA, Grimm C, Galid A, et al. (2004): Polymorphisms of the endothelial nitric oxide synthase gene in women with vulvar cancer. Gynecol Oncol. 93(3):686-90.

Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. (2013): Effects of the T-786C, G894T, and Intron 4 VNTR (4a/b) polymorphisms of the endothelial nitric oxide synthase gene on the risk of prostate cancer. Urol Oncol. 31(7):1132-40.

Shankarishan P, Borah PK, Ahmed G, Mahanta J. (2014): Endothelial nitric oxide synthase gene polymorphisms and the risk of hypertension in an Indian population. Biomed Res Int. 2014:793040.

Toledo JC Jr, Augusto O. (2012): Connecting the chemical and biological properties of nitric oxide. Chem Res Toxicol. 25(5):975-89.

Tontini GE, Vecchi M, Pastorelli L, Neurath MF, et al. (2015): Differential diagnosis in inflammatory bowel disease colitis: State of the art and future perspectives. World J Gastroenterol. 21(1):21-46.

Winawer S, Fletcher R, Rex D, Bond J, et al. (2003): Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. Gastroenterology; 124: 544-560.

Yagihashi N, Kasajima H, Sugai S, Matsumoto K, et al. (2000): Increased in situ expression of nitric oxide synthase in human colorectal cancer. Virchows Arch. 436(2):109-14.

Yang Y, Du K, Liu Z, Lu X. (2014): Endothelial nitric oxide synthase (eNOS) 4b/a gene polymorphisms and coronary artery disease: evidence from a meta-analysis. Int J Mol Sci. 15(5):7987-8003.

Yeh CC, Santella RM, Hsieh LL, Sung FC, et al. (2009): An intron 4 VNTR polymorphism of the endothelial nitric oxide synthase gene is associated with early-onset colorectal cancer. Int J Cancer.124:1565-71.

Zhang L, Chen LM, Wang MN, Chen XJ, et al. (2014): The G894t, T-786c and 4b/a polymorphism in Enos gene and cancer risk: a meta-analysis. J Evid Based Med. 7(4):263-9.

Zhang MX, Zhang C, Shen YH, Wang J, et al. (2008 a): Effect of 27nt small RNA on endothelial nitric-oxide synthase expression. Mol Biol Cell, 19:3997-4005.

Zhang MX, Zhang C, Shen YH, Wang J, et al. (2008 b): Biogenesis of short intronic repeat 27-nucleotide small RNA from endothelial nitric-oxide synthase gene. J Biol Chem, 283:14685-14693.

Zhuang T, Chelluboina B, Ponnala S, Velpula KK, et al. (2013): Involvement of nitric oxide synthase in matrix metalloproteinase-9-and/or urokinase plasminogen activator receptor-mediated glioma cell migration. BMC Cancer. 13:590.

الملخص العربي

دراسة مبدئية عن التعدد الجيني في الانزيم البطاني المصنع لأوكسيد النيتروجين والقابلية لسرطان القولون المصاحب لالْتِهاب القولونِ التَّقَرُّحِيُّ

يعتبر سرطان القولون من اخطر مضاعفات مرض الالْتِهاب القولونِ التَّقَرُّحِيُّ المزمن. وعليه فقد اهتمت هذه الدراسة بأثر التعدد الجيني لجين الانزيم البطاني المصنع لأوكسيد النيتروجين على زيادة خطورة التعرض لسرطان القولون المصاحب لالْتِهاب القولونِ التَّقَرُّحِيُّ. اجري هذا البحث علي شخص مقسمين بالتساوي على ٣ مجموعات : احداهم تشمل مرضى بسرطان القولون القولون المصاحب لالْتِهاب القولونِ التَّقَرُّحِيُّ والاخري تشمل مرضي الْتِهاب القولونِ التَّقَرُّحِيُّ بالاضافة لمجموعة ضابطة. تمت دراسة التحور الجيني في عينات الدم بواسطة استخدام البلمرة الجزيئية ذات التفاعل التسلسلي كما تم قياس مستوى أوكسيد النيتروجين في البلازما عن طريق محلل الطيف الضوئي. وقد اثبتت النتائج الاولية وجود فروق ذو دلالة احصائية بين حاملي التعدد الجيني aa وab عند مقارنتهم بحاملي التعدد الجيني bb في معدل الاصابة بسرطان القولون المصاحب لالْتِهاب القولونِ التَّقَرُّحِيُّ، بينما لايوجد وجود فروق ذو دلالة احصائية عند مقارنة مرضي الْتِهاب القولونِ التَّقَرُّحِيُّ بالمجموعة الضابطة. كما وجد ارتفاع مستوى أوكسيد النيتروجين في البلازما عند ان حاملي التعدد الجيني aa وab من جين الانزيم البطاني المصنع لأوكسيد النيتروجين في مجموعتي مرضي الْتِهاب القولونِ التَّقَرُّحِيُّ ومجموعة سرطان القولون المصاحب له. وقد خلص البحث الى استنتاجات اولية ان حاملي التعدد الجيني aa وab من جين الانزيم البطاني المصنع لأوكسيد النيتروجين قد يكون عندهم قابلية اكثر لحدوث سرطان القولون المصاحب لالْتِهاب القولونِ التَّقَرُّحِيُّ عند مقارنتهم بحاملي التعدد الجيني bb ويوصي البحث باجراء التجربة على نطاق اوسع للتحقق من هذه النتائج الاولية. ذا المرض .