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STUDIES ON LEVELS OF SOME COLORECTAL CANCER ASSOCIATED ANTIGENS/MARKERS IN NEWLY DIAGNOSED COLORECTAL CANCER PATIENTS WITH DIFFERENT ABO BLOOD GROUPS IN YENAGOA

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ABSTRACT
The study on levels of some colorectal cancer associated antigens/markers in newly diagnosed colorectal cancer patients with different ABO blood group was carried out at Federal Medical Centre, Yenagoa. A total of one hundred subjects with different ABO blood groups comprising fifty newly diagnosed colorectal cancer patients (16 of group A, 20 of group B and 14 of group O) and fifty control subjects (17 of group A, 18 of group B and 15 of group O), within the age bracket of 40-70 years were recruited for the study. Samples of blood were collected, prepared by standard methods and sera stored at -20^{o}c prior to use. Analysis was done using immunoturbidimetric assay, enzyme linked immunosorbent assay (ELISA) and gas chromatography-mass spectrometry techniques. Data obtained were subjected to statistical analysis using one way analysis of variance (ANOVA). The results obtained showed that there was a significant increase ($p<0.05$) in CEA value of male CRC group A, female CRC group A, male CRC group B, female CRC group B, male CRC group O and female CRC group O when compared to their control subjects respectively. The Butyric acid of male CRC group A, female CRC group A, male CRC group B, female CRC group B, male CRC group O and female CRC group O decreased significantly ($p<0.05$) when compared to their control subjects respectively. There was a significant decrease ($p<0.05$) in Propionic acid of male CRC group A, female CRC group A, male CRC group B, female CRC group B, male CRC group O and female CRC group O when compared to their control subjects respectively. A significant decrease ($p<0.05$) was observed in Interleukin-6 of male CRC group A and female CRC group A when compared to their control subjects. The Interleukin-8 showed a significant difference ($p<0.05$) in male CRC of both group B and O when compared to their control subjects. The patients of blood group B have the highest value in CEA followed by blood group O and blood group A patients respectively. Similarly, it was observed that patients with blood group B have the lowest values in short chain fatty acids followed by blood group O and blood group A patients. This could predict a more survival rate for colorectal cancer patients with blood group A and less survival rate for blood group B colorectal cancer patients. Therefore, this study concludes that there are observable changes in the CEA and short chain fatty acids of newly diagnosed CRC patients with different ABO blood groups attending Federal Medical Centre, Yenagoa.

KEYWORDS: Colorectal Cancer, ABO blood group, Interleukin, Carcinoembryonic Antigen, Short Chain Fatty Acids.

1. INTRODUCTION
Cancer is defined as a state whereby some cells begin to grow out of natural processes in particular conditions.[1]Some of the signs and symptoms of cancer includes blood in the stool, weight loss, a change in bowel movements and feeling tired all the time.[2] According to Babai,[3] in Iran, cancer is acknowledged as the third foremost cause of death after heart disease and accidents as major causes of death in humans have now moved from infectious diseases to non-communicable ones.

According to National Cancer Institute, colorectal cancer (CRC), which is also called bowel cancer and/or colon cancer, is the development and multiplication of cancer from the colon or rectum (parts of the large intestine).[2] Colorectal cancer (CRC) is one of the main causes of death due to tumorous condition in Western societies and the occurrence of CRC is increasing worldwide.[4] The

predominance of CRC is spurred by dietary factors, demographics, age, lifestyle and family history.[5] Recently, some researchers have identified interleukin-6 (IL-6) in CRC pathogenesis. Various authors have extensively researched about interleukin-6 with various findings. Naka[6] described IL-6 as a pleiotropic cytokine with a vast biological effects which includes autoimmune, cancerogenic and inflammatory. Chung and Chang,[7] showed that serum level of IL-6 is elevated in CRC patients and corresponded with the size of the tumor. Apart from IL-6, IL-8 is also a recognized pro-inflammatory cytokine and significant chemo-attractant factor for leukocytes. Furthermore, according to Baggiolini and Clark-Lewis,[8] IL-8 was revealed to contribute to the development of cancer through its ability as a motogenic, angiogenic and mitogenic factor. Moreover, Kaminska[9] established a rise in cancer tissue and serum IL-8 levels in CRC patients.

Carcinoembryonic antigen (CEA) is well known to be one of the most extensively used tumor markers universally. Various articles have suggested its main claim to mostly as diagnostic profile for gastrointestinal cancers, mostly in colon adenocarcinoma. The measurement of serum CEA has been widely used for postoperative surveillance because of the frequent increase in serum CEA level several months before recurrence is detected on conventional imaging studies.[10] The sensitivity and specificity of serum CEA for the detection of recurrence are thus low because of the irregularities of CEA recurrence in CRC patients.[10]

Various reports have suggested the risk of developing CRC to be associated with diminished production of short chain fatty acids (SCFAs) due to alterations in the intestinal microbiota. Furthermore, some dietary factors like secondary bile acids and ingested fat have been reported to promote tumors a contrast to fiber which has been shown to induce activities against tumors.[11] Short-chain fatty acids (SCFA) which are the main secondary products of fiber fermentation in the gastrointestinal tract have been demonstrated to prompt inhibition of growth and terminal differentiation in a wide range of human colon cancer cell lines.[12] Colonocytes have been shown to use butyrate, propionate and acetate which are the major SCFAs as energy sources a disparity to transformed CRC cells which chiefly experience aerobic glycolysis. CRC cells show increased sensitivity to SCFAs, in comparison to normal colonocytes, thus implying they play a key role in cell homeostasis.

The ABO blood types are defined by carbohydrate moieties displayed on the surface of red blood cells and attached to a protein backbone, known as the H antigen.[13] They are medically the most relevant blood types. The ABO antigens are highly expressed on the surface of epithelial cells of the gastrointestinal, urogenital tract, sensory neurons, platelets and bronchopulmonary in addition to their appearance on the red blood cells surface.[13] The term histo-blood group ABO is mostly used to show the ubiquitous distribution of ABO antigens. It is biologically plausible that the clinical relevance of the ABO system could go beyond immune-haematology, transfusion and transplantation medicine. Modifications in intercellular adhesion, immune-surveillance and membrane signaling, as a result of alterations in surface glycoconjugates could have important implications for the growth of cancer.[14] This finding has induced a great amount of research that examined the relationship between ABO blood type and risk of cancer.

Colon cancer been the most prevalent gastrointestinal cancer is the main cause of death in developed and developing countries like Nigeria.[15] Nevertheless, it is acknowledged recently that gene abnormalities and genetic factors are a major contributor especially at young ages.[16] One of the most crucial and absolute factors in this respect is the individuals genetic background and blood type. This is because blood group has an effect on behaviors and plays a major role in illness and mental health.[17]

A better knowledge of the environmental and genetic factors affecting colorectal cancer can improve the prevention, treatment and recovery process. Other research has shown blood group, as a major genetic factor, to play an important role in the occurrence and prevalence of cancer.

A genome-wide association study (GWAS) recently revealed the involvement of genetic variation in the ABO locus of 9q34 to pancreatic carcinogenesis[18] but such notable association has not been identified for CRC. There are little or no studies on the association of ABO antigen expression either on erythrocyte or tumor cell and genetic factors in colorectal cancer, hence the aptness of this study. This research was aimed to evaluate the levels of some colorectal cancer associated antigens/markers in newly diagnosed colorectal cancer patients with different ABO blood group in Yenagoa.

2. MATERIALS AND METHODS

A. Study Area

This study was conducted at the Federal Medical Centre Yenagoa, in Bayelsa State. Yenagoa is the capital and seat of power of Bayelsa State in South South Nigera. It has an area of 706km^2 and official human population figure of 352,285 humans according to 2006 census data.[19] It lies on latitude 4^{0}55'29" N, longitude 6^{0}15'51" E. The city enjoys an annual average rainfall of 2899mm/annum and a relative humidity of 90%.

B. Advocacy, Mobilisation, and Pre-Survey Contacts

A letter of introduction from my department and an application letter were presented to the research ethics committee of Federal Medical Centre, Yenagoa, Bayelsa State. Ethical approval was granted and obtained. Before commencement, the informed consents of the participants were also obtained before enrollment into

the study. They were assured of confidentially of the information obtained from them during and after the study.

C. Experimental/Study Design .

A total of 100 subjects with different ABO blood group between the ages of 40-70 (both male and females) were recruited for this study; this included 50 newly diagnosed CRC patients (16 of group A, 20 of group B and 14 of group O) who are attending gastroenterology/surgical clinic at the gastroenterology department of the F.M.C Yenagoa and 50 apparently healthy subjects with different ABO blood group (17 of group A, 18 of group B and 15 of group O) and no history of colorectal cancer who are age-matched with the test subjects which will serve as controls. The patients were those who have had a proper diagnosis of colon cancer following a lower gastrointestinal colonoscopy

D. Criteria for Selection

i. Inclusion Criteria

a. Newly diagnosed patients with colorectal cancer (between 0 to 6 months).
b. Patients between the ages of 40-70years.
c. Those who gave their consent for the research.

ii. Exclusion Criteria

a. Patients below the age of 40 years
b. Populations with personal history of inflammatory bowel disease, family history of CRC, and known genetic susceptibility syndromes (e.g., Lynch Syndrome).

E. Data Collection

Data was collected through use of questionnaire and biochemical analysis of blood sample.

Questionnaire

Structured questionnaire was employed for data collection. The questionnaire was designed for demographic data such as age, sex, marriage status, cigarette and alcohol consumption (yes/no), prescribed drugs, medical history e.t.c.

The essence was to achieve accurate, relevant, timely and unbiased result.

F. Sample Collection

Six (6) millilitre of blood sample was collected aseptically from all subjects by venipuncture technique from the cubital fossa. Two (2) milliliters was discharged into labeled EDTA bottle while four (4) millilitres into well labeled plain test tubes. The samples in the labeled plain were allowed to clot and then all centrifuged at 4,000 rpm for 10 minutes. The sera was then transferred into properly labeled plain tubes and stored at -20^{0}C until assayed.

G. Laboratory Analysis

a. Determination of Carcinoembryonic Antigen by Immunoturbidimetric Assay (RayBiotech Life, 3607 parkway lane, suite 200 Peachtree Corners, GA 30092 united states).

Procedure:

The manufacturer's instructions were strictly adhered to.

b. Determination of Butyric Acid by Enzyme-Linked Immunosorbent Assay (Elabscience Biotechnology, 14780 Memorial Drive, Suite 216, Houston, Texas 77079).

Procedure:

The manufacturer's instructions were strictly adhered to.

c. Determination of Interleukin 6 (IL-6) by Enzyme-Linked Immunosorbent Assay **(**Elabscience Biotechnology, 14780 Memorial Drive, Suite 216, Houston, Texas 77079).

Procedure:

The manufacturer's instructions were strictly adhered to.

d. Determination of Interleukin 8 (IL-8) by Enzyme-Linked Immunosorbent Assay **(**Elabscience Biotechnology, 14780 Memorial Drive, Suite 216, Houston, Texas 77079).

Procedure:

The manufacturer's instructions were strictly adhered to.

e. Determination of Propionic Acid by Gas Chromatography-Mass Spectrometry (Molbase Biotechnology, Floor 4-5, Building 12, no 1001, North Qingzhou Road, Xuhui District, Shanghai, China).

Procedure

The manufacturer's instructions were strictly adhered to.

H. Statistical Analysis

All values were expressed as mean ± Standard deviation (SD) using SPSS version 21.0. All data generated from this study were statistically analyzed using one way Analysis of variance (ANOVA). Results were displayed in tables. A correlation analysis was also considered. The 5% (<0.05) level of significance was adopted for significance.

3. RESULTS

Table 4.1: Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed male CRC patients and male healthy individuals (controls) of blood group A.

Parameters	CRC Patients n=50	Control Subjects n= 50	F-value	P-value
CEA (ng/ml)	30.84±1.95	1.98±0.15	12.862	0.000
Butyric Acid (µg/ml)	1.26±0.44	6.04±0.21	1.710	0.000
Propionic Acid (µg/ml)	1.98±0.17	8.91±0.40	31.992	0.000
Interleukin-6 (pg/ml)	4.37±1.01	6.04±0.71	3.153	0.002
Interleukin-8 (pg/ml)	9.35±1.34	10.25±1.05	0.008	0.156

Table 4.2 Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed female CRC patients and female healthy individuals (controls) of blood group A.

Parameters	CRC Patients n=50	Control Subjects n=50	F-value	P-value
CEA (ng/ml)	34.42±1.13	1.98±0.99	16.195	0.000
Butyric Acid (µg/ml)	1.83±0.37	6.13±0.47	0.740	0.000
Propionic Acid (µg/ml)	1.92±0.34	8.72±0.37	0.791	0.000
Interleukin-6 (pg/ml)	4.79±0.85	6.93±0.87	0.006	0.000
Interleukin-8 (pg/ml)	10.57±1.12	10.04±0.66	1.574	0.271

Table 4.3: Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed male CRC patients and male healthy individuals (controls) of blood group B.

Parameters	CRC Patients n=50	Control Subjects n= 50	F-value	P-value
CEA (ng/ml)	87.57±3.42	3.88±0.57	5.318	0.000
Butyric Acid (µg/ml)	0.17±0.07	6.16±0.75	46.820	0.000
Propionic Acid (µg/ml)	1.03±0.12	8.21±0.52	16.738	0.000
Interleukin-6 (pg/ml)	4.88±0.39	4.79±0.45	0.462	0.692
Interleukin-8 (pg/ml)	10.17±0.69	11.59±0.82	0.631	0.002

Table 4.4 Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed female CRC patients and female healthy individuals (controls) of blood group B.

Parameters	CRC Patients n=50	Control Subjects n= 50	F-value	P-value
CEA (ng/ml)	128.55±6.41	3.37±0.76	4.870	0.000
Butyric Acid (µg/ml)	0.22±0.56	6.57±0.66	12.848	0.000
Propionic Acid (µg/ml)	1.02±0.10	7.86±0.48	48.696	0.000
Interleukin-6 (pg/ml)	5.08±0.37	5.09±0.53	3.097	0.928
Interleukin-8 (pg/ml)	10.89±0.84	11.42±1.06	0.105	0.215

Table 4.5 Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed male CRC patients and male healthy individuals (controls) of blood group O.

Parameters	CRC Patients n=50	Control Subjects n= 50	F-value	P-value
CEA (ng/ml)	0.62±0.08	1.72±0.73	0.140	0.000
Butyric Acid (µg/ml)	0.62±0.08	6.28±0.64	8.505	0.000
Propionic Acid (µg/ml)	1.69±0.33	8.02±0.34	0.047	0.000
Interleukin-6 (pg/ml)	5.02±0.12	4.96±0.48	10.053	0.768
Interleukin-8 (pg/ml)	10.16±0.55	11.53±0.63	0.195	0.000

Table 4.6 Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed female CRC patients and female healthy individuals (controls) of blood group O.

Parameters	CRC Patients n=50	Control Subjects n= 50	F-value	P-value
CEA (ng/ml)	70.27±1.40	1.82±0.36	6.289	0.000
Butyric Acid (µg/ml)	0.67±0.05	6.43±0.73	0.000	0.000
Propionic Acid (µg/ml)	1.75±0.38	8.01±0.42	10.468	0.000
Interleukin-6 (pg/ml)	5.05±0.15	4.94±0.45	6.555	0.582
Interleukin-8 (pg/ml)	8.65±4.28	11.26±0.59	5.657	0.110

Table 4.7 Mean ± SD values of Levels of Carcinoembryonic antigen, Butyric acid, Propionic acid, Interleukin-6, and Interleukin-8 in newly diagnosed CRC patients among the various blood groups.

Parameters	Group A	Group B	Group O	F-value	P-value
CEA (ng/ml)	32.75±2.38	112.16±21.27	71.26±1.49	140.953	0.000
Butyric Acid (µg/ml)	1.56±0.49	0.20±0.07	0.64±0.07	103.338	0.000
Propionic Acid (µg/ml)	1.95±0.27	1.02±0.11	1.72±0.34	70.180	0.000
Interleukin-6 (pg/ml)	4.59±0.92	4.99±0.38	5.02±0.13	2.750	0.075
Interleukin-8 (pg/ml)	10.00±1.34	10.60±0.84	9.46±2.89	1.680	0.198

Table 4.8 Post Hoc (Turkey LSD Test) Comparison of Carcinoembryonic antigen (CEA), Butyric acid (C4), Propionic acid (C3), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) in newly diagnosed CRC patients between the various blood groups.

Blood groups	CEA (ng/ml)	C3 (µg/ml)	C4(µg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
Group A vs B	0.000[a]	0.000[a]	0.000[a]	0.044[a]	0.326[b]
Group A vs O	0.000[a]	0.015	0.000[a]	0.051[b]	0.422[b]
Group B vs O	0.000[a]	0.000[a]	0.000[a]	0.883[b]	0.077[b]

The [a]mean difference is significant at the 0.05 level.
[b]The mean difference is not significant at the 0.05 level.
N.B: C3= propionic acid
C4=butyric acid

Table 4.9 Pearson correlation of serum Carcinoembryonic antigen (CEA) with Butyric acid (C4), Propionic acid (C3), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) in newly diagnosed CRC patients.

Dependent Variables	n	r-value	P-value
Butyric Acid (µg/ml)	50	-0.794**	0.000
Propionic Acid (µg/ml)	50	-0.810**	0.000
Interleukin-6 (pg/ml)	50	0.313*	0.030
Interleukin-8 (pg/ml)	50	0.220	0.133

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

4. DISCUSSION

The findings of the study revealed that carcinoembryonic antigen (CEA) was significantly increased ($p<0.05$) in the various ABO blood group of newly diagnosed colorectal cancer patients (CRC) when compared with the control. This observed significant increase was consistent for both male and female newly diagnosed colorectal cancer patients. This observation could be linked to various publications that suggested that as CEA exist in normal colorectal mucosa, increased proliferation of cells would result to increased concentration due to expression of carcinoembryonic antigen in colon mucosa cells[20] Furthermore, Thomas[21] reported that carcinoembryonic antigen (CEA) are involved in increased metastatic potential, intercellular adhesion and protection against anoikis (apoptosis associate with cell detachment from the extracellular matrix) therefore, carcinoembryonic antigen (CEA) would be increased in condition of increased cellular activities such as cancer. In addition, carcinoembryonic antigen (CEA) has been extensively used as a special tumor marker of colorectal cancer and there has been strong association suggesting an inter play with the dysregulation of the enzymatic activity of the ABO glycosyltransferase, which are specifically involved in the processes of intercellular adhesion and cellular membrane signaling as well as in the immune response to the host.[22] The alteration of these surface molecules promotes the process of malignancy.[23]

The observed findings of this study showed a significant decrease ($p<0.05$) in the concentration of the short chain fatty acid (butyric acid and propionic acid) in the various

ABO blood groups of newly diagnosed colorectal cancer patient when compared with the control. The observed significant decrease was seen in both male and female CRC patients. The suggested possible mechanism is alterations of the intestinal microbiota by cancer proliferation process which directly induces decreased production of short chain fatty acids (butyric acid and propionc acid). However, dietary factors such as secondary bile acids and ingested fat have been shown to promote tumors, whereas short chain fatty acids which are the major secondary products of fibre fermentation in the gastrointestinal tract and have been shown to induce the inhibition of growth and terminal differentiation in a variety of human colon cancer cell lines.[24] These short chain fatty acids have also been shown to promote healthy colonic epithelial cells.[25]

Furthermore, the findings of the study also revealed a significant ($p<0.05$) decrease in the concentration of interleukin-6 for both male and female CRC patients of blood group A when compared with the control. There was also a significant decrease ($p<0.05$) in the concentration of interleukin-8 for the male CRC patients of blood group B and male CRC patients of blood group O when compared with their various control groups respectively. Similarly, a decrease in the concentration of interleukin-8 for the female CRC patients of blood group B and female blood group O was observed but statistically non-significant ($p>0.05$). Although these findings are not in consonance with reports by Kollma[26] and that of Chung and Chang,[7] where they reported Interleukin-6 and interleukin-8 as an important tumor promoting factor in colorectal cancer and thus are expressed in colon cancer cells. There is a possibility of increased production of anti-inflammatory protein in the early stage of cancer which suppressed the production of interleukin-6 and interleukin-8.

Comparison of the different ABO blood groups of newly diagnosed CRC patients against the various antigens/markers showed that CEA, propionic acid and butyric acid were all statistically significant ($p<0.05$), whereas interleukin-6 and interleukin-8 were statistically non-significant ($p>0.05$). These observed statistically significant differences were clinically significant at 95% confidence level.

Pearson correlation of the serum CEA with propionic acid, butyric acid, interleukin-6 and interleukin-8 showed some variations. This variation could probably be attributed to the difference in their mechanism of action. The serum propionic acid ($p=0.000$, $r= -0.794^{**}$) and butyric acid ($p=0.000$, $r= -0.810^{**}$) showed a significant negative correlation with CEA in newly diagnosed CRC patients. Furthermore, the serum interleukin-6 ($p=0.030$, $r= 0.313^{*}$) showed moderate positive relationship while interleukin-8 ($p=0.133$, $r= 0.220$) was not significantly correlated with CEA in newly diagnosed CRC patients.

5. CONCLUSION

The findings of this study revealed significant changes in the antigens/markers of newly diagnosed CRC patients with different ABO blood group. CEA levels increased in all the ABO blood groups of CRC patients and there was also a decrease in short chain fatty acids (butyric acid and propionic acid) of CRC patients with different ABO blood group. There was also a strong negative correlation between CEA and short chain fatty acid in the studied newly diagnosed CRC patients. Interleukin-6 showed moderate positive association while interleukin-8 was not significantly correlated with CEA. The patients with blood group B have the highest value in CEA followed by blood group O and blood group A patients respectively. Similarly, it was observed that patients with blood group B have the lowest values in short chain fatty acids followed by blood group O and blood group A patients. This could predict a more survival rate for colorectal cancer patients with blood group A and less survival rate for blood group B colorectal cancer patients. Therefore, this study concludes that there are observable changes in the CEA and short chain fatty acids of newly diagnosed CRC patients with different ABO blood groups attending Federal Medical Centre, Yenagoa.

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REFERENCES

1. Mohebbi, Z., Amiri, M., SomeaSaraee, H.J. Blood group, survival key. Tehran: Company of Karafarinan Farhang & Honar, 2004.
2. *National Cancer Institute. "Defining Cancer "Archived from the original, 2014; 25.*
3. Babai, M., Mousavi, S., Malek, M., Danaie, N., Jandaghi, J.,Tousi, J. Survey of cancer incidence during a 5-year (period in Semnan province [InPersian]. *Koomesh Journal*, 2005; 6(3): 237–244.
4. Pohl, A., Zhang, W., Ning, Y., Manegold, P.C., Lurje, G., Lenz, H.J. Targeting metastatic colorectal cancer in 2008: a long way from 5-FU. *Oncology*, 2008; 22: 456-462.
5. Benito, M., Diaz-Rubio, E. Molecular biology in colorectal cancer. *ClinicalTranslational Oncology*, 2006; 8: 391-398.
6. Naka, T., Nishimoto, N., Kishimoto, T. The paradigm of IL-6: from basic science to medicine. *Arthritis Research and Therapy, 2002;* 4: 233-242.
7. Chung, Y.C., and Chang, Y.F. Serum interleukin-6 levels reflect the disease status of colorectal cancer. *Surgical Oncolology, 2003;* 83: 222-226.

8. Baggiolini, M., Clark-Lewis, I. Interleukin 8, a chemotactic and inflammatory cytokine. *FEBS Letters, 1992;* 307: 97-101.
9. Kaminska, J., Nowacki, M.P., Kowalska, M. Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I - an independent prognostic factor. *Tumor Biology*, 2005; 26: 186-194.
10. Meyerhardt, J.A., Mangu, P.B., Flynn, P.J., Korde, L., Loprinzi, C.L., Minsky, B.D., Petrelli, N.J., Ryan, K., Schrag, D.H., Wong, S.L, Benson, A.B. 3rd: Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. *Journal of Clinical Oncology, 2013;* 31: 4465-4470.
11. Mcintyre, A., Gibson, P. R., Young, G. P. Butyrate production from dietary fiber and protection against large bowel cancer in a rat model. *Gut, 1993;* 34: 386–391.
12. Whitehead, R. H., Young, G. P, Bhathal, P. S. Effects of short chain fatty acids on a new human colon carcinoma cell line (LIM 1215). *Gut, 1986;* 27: 1457–1463.
13. Hakomori, S. Antigen structure and genetic basis of histo-blood groups A, B and O: their changes associated with human cancer. *Biochimica BiophysicaActa, 1999;* 1473(1): 247–266.
14. Zhang, S., Zhang, H.S., Cordon-Cardo, C., Reuter, V.E., Singhal, A.K., Lloyd, K.O. Selection of tumor antigens as targets for immune attack using immunohistochemistry: Blood group-related antigens. *InternationalJournal of Cancer*, 1997; 73(1): 50–56.
15. Tazhibi, M., Khalil, T. H., Adeli, M., Abdar, N., Loghmani, L., Maghroun, M. Association between major dietary patterns and risk of colon cancer. *Journal of Isfahan Medical School, 2011;* 28: 122-128.
16. Jasperson, K.W., Tuohy, T.M., Neklason, D.W., Burt, R.W. Hereditary and familial colon cancer. *Gastroenterology, 2010;* 138(6): 2044–2058.
17. Ali, K. J., Seyyed, M.K., Mohammad-Rafi, B., Seyyed, H.K. The relationship between blood group and colon cancer in Shiraz Namazi Hospital durin *Jundishapur Journal of Clinical Disease Care*, 2017; 7(10): 459-474.
18. Amundadottir, L., Kraft, P., Stolzenberg-solomon, R.Z., Fuchs, C.S., Petersen, G.M., Arslan, A.A. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nature Genetics*, 2009; 41(9): 989-990.
19. National Population Commission. Report of Nigeria's National Population Commission on the 2006 Census. *Population and Development Review, 2006;* 33(1): 206-210.
20. Bhatnagar, J., Tewari, H., Bhatnagar, M., Austin, G.E. Comparison of carcinoembryonic antigen in tissue and serum with grade and stage of colon cancer. *Anticancer Research*, 1999; 19: 2181–2188.
21. Thomas, P., Forse, R.A., Bajenova, O. Carcinoembryonic antigen and its receptor hnrnp m are mediators of metastasis and the inflammatory reponse in the liver. *Clinical of Experimental Metastasis*, 2011; 28: 923-932.
22. Hakomori, S. Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. *Advances inExperimental Medicine and Biology*, 2001; 491: 369-402.
23. Franchini, M., Crestani, S., Frattini, F. ABO blood group and von Willebrand factor: biological implications. *Clinical Chemistry andLaboratory Medcine*, 2014; 52: 1273-1276.
24. Lupton, J.R. Microbial Degradation Products Influence Colon Cancer Risk: the Butyrate Controversy. *Journal of Nutrition, 2004;* 134: 479–482.
25. Vanhoutvin, S.A., Troost, F.J., Hamer, H.M., Lindsey, P.J., Koek, G.H., Jonkers, D.M., Kodde, A., Venema, K., Brummer, R.J. *Bereswill S (ed.).* "Butyrate-induced transcriptional changes in human colonic mucosa". *PLOS ONE, 2009; 4(8): 6759-6760.*
26. Kollmar, O., Scheuer, C., Menger, M.D., Schilling, M.K. Macrophage inflammatory protein-2 promotes angiogenesis, cell migration, and tumor growth in hepatic metastasis. *Annals of Surgical Oncology*, 2006; 13: 263–275.