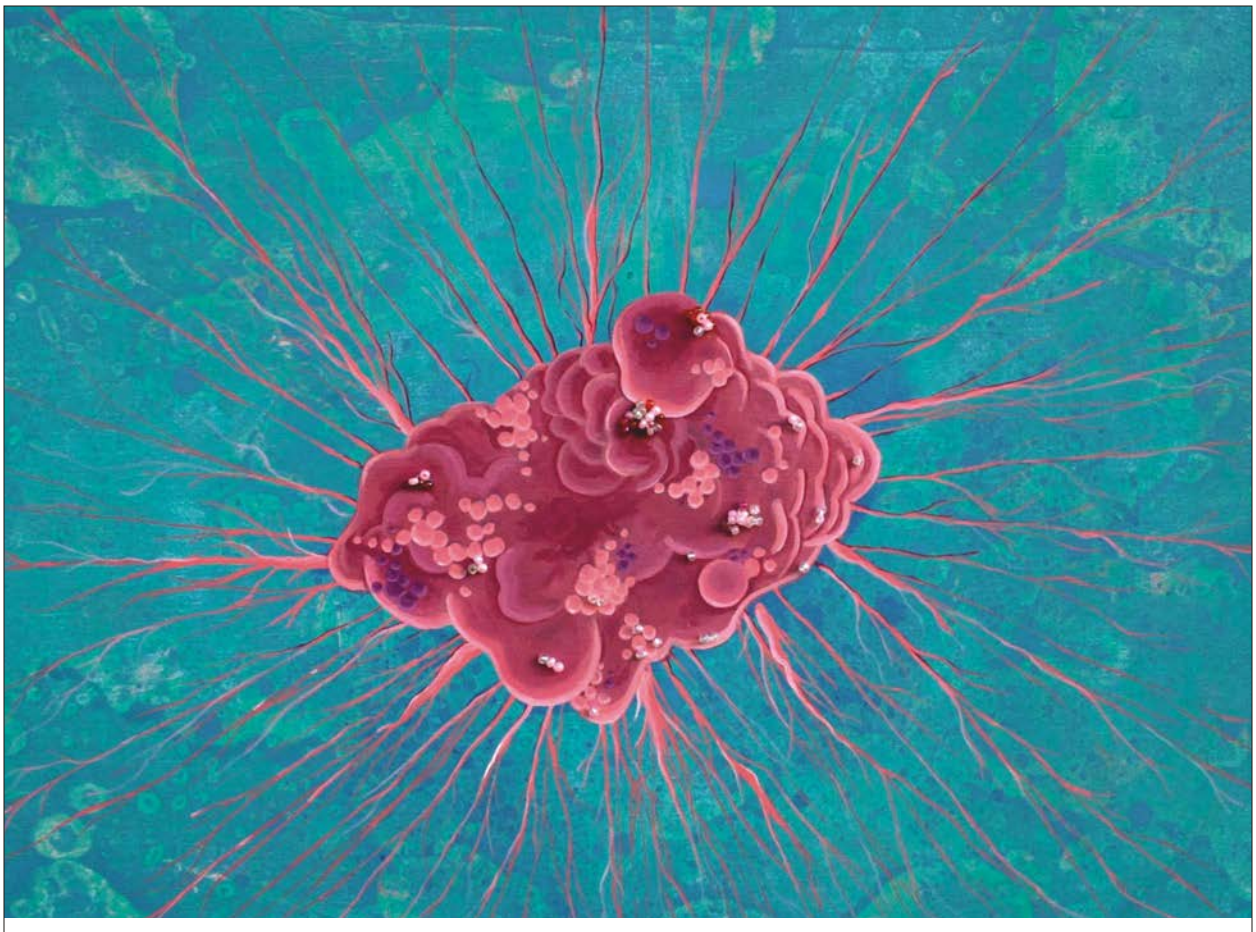

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ABO Blood Group and Risk of Malignancies in Egyptians

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ABSTRACT

There is growing evidence that the ABO blood group system may play a role in disease etiology. Studies conducted several decades ago, have demonstrated a relationship between inherited human ABO and Rhesus blood groups and risk of various malignancies. However, these findings are inconsistent and contradictory. The objective was to perform analysis of ABO and Rhesus blood antigens distribution among patients with various cancers including breast, hepatocellular, pancreatic, gastric, skin, lung cancers, leukemia and lymphoma in Dakahlia, Egypt and to assess their potential role in carcinogenesis. A total of 1131 cancer patients (age 63.8±9.3, female/male, 215/916) and another 1200 healthy controls (age 48.9±11.3, female/male, 348/852) were enrolled in this study. ABO blood groups were determined using Tube method and Gel method. The anti-TF IgG level and Von Willebrand factor were determined by enzyme-linked immunosorbent assay. The distribution of blood type A was significantly higher among cancer patients than among healthy controls (39.35 vs. 33.75%, $p = 0.014$), whereas, the distributions of other blood types were similar between cases and controls ($p>0.05$). Most cancers, especially gastrointestinal tumors were dominated by male gender independent of age. Blood group A was associated with significantly higher risk for malignancy including hepatocellular carcinoma, pancreatic and breast cancers while biliary and esophageal cancer risk was significantly associated with blood type B. We suggest that blood group A may elevate risk of cancer and may play a role in its development.

Key words: ABO and Rhesus blood group, cancer, hepatocellular carcinoma

INTRODUCTION

ABO blood group is one of the most important blood group antigens that must be correctly identified prior to transfusion and transplantation procedures. ABO genotyping is commonly used in cases of an ABO discrepancy between serum and cell typing (Eastlund, 1998). Serological ABO typing is performed using the anti-A and anti-B antisera of polyclonal or monoclonal origin, which can distinguish the four phenotypes (A, B, AB and O) (Garratty, 2000). ABO blood group genes are mapped at 9q34.2 region in which genetic alteration is common in many cancers. Several authors have reported a correlation of blood group antigen expression in tumor with metastasis and prognosis for various human malignancies, such as colon, breast and prostate cancer. The carbohydrates of blood group expressed on metastatic cancer cells surface acts as cell adhesion

molecule (Guleria *et al*., 2005). Blood group antigens, which are the major alloantigens in humans are present not only on the surface of Red Blood Cells (RBC) but also on various human cells and tissues, including the epithelium, sensory neurons, platelets and the vascular endothelium (Storry and Olsson, 2009).

Several reports have suggested an important involvement of ABO blood group system in the development of cardiovascular, oncological and other diseases (Franchini *et al*., 2012). As the majority of human cancers are derived from epithelial cells, changes in blood group antigens are an important aspect of human tumor (Henderson *et al*., 1993). Some blood groups are found to be associated with certain medical conditions. For example, blood group O antigens are associated with increased incidence of peptic ulcer and urinary tract infection. Blood group A antigens are more commons with cancer stomach, salivary gland, colon, ovary, uterus, cervix and bladder. In some tumors, alteration of ABO/Lewis-related antigens is associated with malignant transformation (Liumbruno and Franchini, 2013). The relationship with blood groups and incidence, clinicopathologic parameter and prognosis had been studied in many cancers such as esophagus, cardiac, gastric, lung, colorectal, pancreatic, bone, urinary bladder, ureter, renal, breast, prostate and melanoma (Karakousis *et al*., 1986). Additionally, ABO genes are distributed differently among socioeconomic groups and we know that socioeconomic status is one of the risk factors for disease (Beardmore *et al*., 1983). Numerous reports have documented a relation between susceptibility to cancer and blood group. High incidence of blood group A in various cancers, including neurologic tumors, salivary gland, colon, ovary, kidney and cervix and consistent relation to O blood group in skin and melanoma has been reported (Iodice *et al*., 2010).

The Lewis blood group antigens form terminal carbohydrate structures on cellular surfaces. The molecular structure of the Lewis antigens is related to that of the ABO blood group antigens A, B and O. Their biosynthesis proceeds from common precursors and involves stepwise addition of monosaccharides catalyzed by glycosyltransferases (Kurtenkov *et al*., 1999).

Preclinical studies showed that the expression of selectin ligands sLea and sLex on tumor cells was induced by hypoxia and increased the cellular adhesion of these selected clones to endothelial cells. Subsequent *in vivo* studies demonstrated tumor angiogenesis to be significantly dependent upon E-selectin mediated cell adhesion of tumor cells expressing sLea and sLex to endothelial cells. Furthermore, the adhesion of tumor cells via., sLea and sLex to vascular endothelial cells may facilitate hematogenous metastasis (Qi *et al*., 2010).

Although several studies have demonstrated a prognostic value for the ABO blood group antigens in various malignancies, the results of these studies are controversial and inconsistent suggesting that the biological role of ABH antigens may be disease-specific.

Previous studies underlined the importance of ABO blood group antigens for cancer progression demonstrated that Carbohydrate 19-9 (CA19-9) which is the sialylated Lewis a (sLea) blood group antigen and firstly described by (Koprowski *et al*., 1979) had a prognostic value in patients with resectable and advanced cancers as pancreatic cancer (Ferrone *et al*., 2006). Serum levels of CA19-9 were found to be elevated in 70-80% of patients with Pancreatic Cancer (PC) and therefore, could be used routinely in monitoring the disease course and progression (Wolpin *et al*., 2009).

Hepatocellular Carcinoma (HCC) is the major form of primary liver cancer, the fifth most common cancer and the third leading cause of cancer mortality worldwide, it is one of the most aggressive cancers with well-identified risk factors, including cirrhosis and chronic viral hepatitis B and C (HBV, HCV). However, not all patients with these diseases will develop HCC suggesting that there are other factors within these disease groups that indicate greater or lesser risk. Other

important risk factors and predictors for HCC are older age, male gender and sustained activity of liver disease, cirrhosis, diabetes mellitus, alcohol abuse, obesity and family history of liver cancer. Moreover, in those with chronic HBV infection, viral genotype, HBV DNA level and positive hepatitis B e antigen (HBeAg) have been identified as risk predictors for HCC (Sherman, 2010). Human genetic factors including polymorphisms of Tumor Necrosis Factor-α (TNF-α) and Epidermal Growth Factor (EGF) gene have also been identified to be associated with HCC risk (Abu Dayyeh *et al*., 2011).

No report has evaluated the relationship between the ABO blood groups and cancers in Egypt. The present study attempted to correlate ABO blood group frequency and to assess the utility of ABO blood group as a preclinical tumor marker. Thus, the objectives of this study were to document ABO blood group of patients suffering from malignancies of different organs and systems and to describe the association of different type of malignancies with ABO blood group in Dakahlia, Egypt.

MATERIALS AND METHODS

This is a retrospective hospital-based, case-control study conducted in Mansoura University Hospitals (MUH) from January 2010-2014. A total of 1131 consecutive, newly and confirmedly diagnosed cancer patients, with age ranging from 8-75 years (M±SD; 63.8±9.3), (female/male; 215/916) were enrolled in this study as patient group. Another 1200 (female/male; 348/852) healthy subjects with age ranging from 11-64 years (M±SD; 48.9±11.3) and with no known disease (volunteers, medical students, blood donors, paramedical and health workers) were also randomly included in this study as control group. The study was approved by the Ethical Commission and Institutional Review Board of Mansoura University Hospital in EGYPT. A written informed conscious consent was obtained from all subjects before their participation. The data of age, sex, ABO blood type and pathological status of cancer patients were collected from Oncology center, Faculty of Medicine in Mansoura University. Control subjects were selected among healthy people with no history of cardiovascular disease, cancer, chronic degenerative neurologic disease, chronic obstructive pulmonary disease, hepatitis, allergies in general or alcohol abuse. Documentation of clinical findings included age, gender, family history, type 2 diabetes and clinical types of cancer and ABO blood group.

The inclusion criteria for cases were as follows: Pathologically confirmed diagnosis of any cancer type; Laboratory data available for ABO blood type and diabetes screening and detailed record of disease course and history. The exclusion criteria were the absence of laboratory data on blood types and plasma glucose. The inclusion criteria for controls were the same as those for cases, except for the cancer diagnosis.

Initially, all patients and controls completed a detailed questionnaire regarding diet and habits, submitted to thorough history taking and detailed physical examinations and performed routine radiological and laboratory investigations including, CBC, liver and renal function tests, plasma glucose level, viral markers, tumor markers for specific cancer.

Blood samples were obtained into vacuum tubes containing EDTA (vacutainer, Becton Dickinson, Marseilles, France) from each donor's venous circulation.

ABO and Rhsus blood typing were carried out with tube method and gel method (Rumsey and Ciesielsky, 2000). ABO blood groups were determined using (Diclone antigen A, B and AB monoclonal), IgM antibody for blood grouping. (DiMed AG, Switzerland).

Tube method: One drop of antigen A, B, or D (Eryclone, Diagnostics) was added to the appropriately labeled tube. A five percent suspension of RBC was made in isotonic saline. One drop was added to tubes containing antigen A, B, or D. The contents of the tubes were mixed thoroughly and the tubes were centrifuged for 20 sec at 3400 rpm. Tubes were read macroscopically for agglutination (Giger *et al.*, 2005).

Gel method: Five percent RBC suspension was prepared in diluents (modified bromelin solution for red cell suspensions). Gel cards (Diaclon ID, Diamed AG, Cressier, Switzerland) were used for ABO and Rh typing. Ten μL of RBC suspension were added to the gel micro-tubes containing antigen A, B, D and control reagents, respectively. Fifty μL of donor plasma were added to micro-tubes for reverse ABO group testing. The ID cards were centrifuged at 895 rpm 10 min in the centrifuges (ID-centrifuge). A positive reaction (4+) was determined by the formation of a red line on the gel surface, whereas intermediate reactions were characterized by red agglutinates distributed throughout the gel. With a negative reaction, a compact button of cells formed on the bottom of the micro-tube (Judd *et al.*, 1997).

Lewis blood group phenotype: It was also performed on the saline-washed RBCs by the standard hemagglutination technique using monoclonal anti-Le (a) and anti-Le (b) (Lorne Labs, Reading, Berkshire, UK) according to the manufacturer's instructions. Individuals with Le (a-/b+) were assigned as secretors, those with Le (a+/b-) were assigned as non-secretors and those with Le (a-/b-), i.e., LDN, were assigned unknown secretor status, for whom saliva was used to determine their secretor status by the standard hemagglutination inhibition test.

The Anti-TF IgG: The Anti-TF IgG level was determined by enzyme-linked immunosorbent (ELISA) assay according to the manufacturer's instructions (Desai *et al.*, 1995).

Von willebrand factor (VWF): The Assay Max human VWF ELISA kit is designed for detection of human VWF in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures VWF in less than 5 h according to the manufacturer's instructions. The minimum detectable level of vWf was typically less than 1 μU mL^{-1}. Intra-assay and inter-assay coefficients of variation were 4.9 and 7.6%, respectively. No significant cross-reactivity or interference was observed. Normal human plasma vWf concentration has been reported ranging approximately from 0.3-1.57 lU mL^{-1}. Normal citrated human plasma vWf values are 0.52-1.54 lU mL^{-1} for O blood group subjects and 0.6-2.0 lU mL^{-1} for non-O blood group subjects (Pittet *et al.*, 1997).

Statistical analysis: Data were analyzed using SPSS software (Version 17.0). Quantitative data were expressed as (Mean±SD) and compared using student's t test while qualitative data were expressed as number and percentage. Continuous data are expressed as median (range) and were evaluated by appropriate statistical tests. The chi-square test (x^2) was used as a global test for any relationship and proportions. Dependence analysis was used to calculate associations among the results. Multivariate unconditional logistic regression analyses were performed using all variables significant at $p<0.05$ in the single factor analyses. For each factor, we calculated the adjusted

Odds Ratio (OR) and 95% Confidence Interval (CI) using maximum likelihood estimation. Correlations were evaluated using the Spearman rank correlation coefficient test. Kruskal-Wallis One Way Analysis Of Variance (ANOVA) compares more than two groups. p-value≤0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics in cancer patients versus healthy controls were shown in Table 1. As expected, most cancer patients, especially those with gastrointestinal tumors, were male (81%) and older age than controls, the mean age±standard deviation was 63.8±9.3 years for patients and 48.9±11.3 years for controls (p<0.001). However, thyroid and breast cancers were more prevalent in females than males. Although, not statistically significant, the prevalence of a positive family history of cancer and the presence of type 2 diabetes seemed to be higher in the cancer patients compared with healthy controls.

The associations between cancer types and different risk factors including; sex, age, family history and type 2 diabetes were shown in Table 2. The distribution of these clinical traits among

Table 1: Demographic and clinical characteristics in cancer patients versus healthy controls

	Patients (1131)		Control (1200)				
	N	(%)	N	(%)	OR (95% CI)	p-value	X^2
Sex (F/M)	215/916	19/81	348/852	29/71	1.96 (1.07-1.89)*	<0.001	S
Age (y)							
20	46	4.1	60	5	1 (reference)	< 0.001	S
20-40	79	7	408	34	3.35 (2.57-4.69)		
41-60	724	64	600	50	9.45 (7.17-11.89)		
60	282	24.9	132	11	14.2 (11.07-17.39)*		
Family history (no/yes)	996/135	88/12	1104/96	92/8	1.02 (0.87-1.39)	0.107	ns
Type 2 diabetes (no/yes)	1006/125	89/11	1092/108	91/9	1.25 (1.08-1.69)	0.015	ns

NS: Non significant, S: Significant

Table 2: Associations between different risk factors and cancer types for each cancer, multivariable logistic regression analysis was performed versus healthy control

	Sex		Age		Family history		Diabetes	
Cancer type	[1]OR	95% CI	OR	95% CI	OR	95% CI	OR	95%CI
HCC	3.22	2.35-4.38	1.21	1.19-1.23	6.32	3.33-12.62	0.29	0.15-0.55
Esophageal	3.12	2.41-4.29	1.32	1.21-1.40	6.21	3.12-11.81	0.39	0.24-0.65
Gastric	1.92	1.41-2.44	1.13	1.08-1.13	5.37	2.99-10.70	2.07	1.46-3.27
Biliary	0.91	0.68-1.11	1.09	1.02-1.12	6.19	3.23-11.92	2.23	1.89-3.32
Pancreatic	1.23	0.96-1.57	1.08	1.00-1.11	4.98	3.22-7.710	2.99	1.46-3.34
Thyroid	0.46	0.41-0.59	1.01	0.99-1.21	2.64	1.03-5.970	1.16	0.55-1.32
Breast	0.48	0.44-0.61	1.02	0.97-1.01	2.46	1.41-5.730	0.99	0.75-1.96
Lymphoma	1.19	0.91-1.47	0.93	0.95-1.12	3.11	1.32-6.770	1.11	0.88-1.43
Skin	1.21	0.93-1.39	1.07	1.02-1.15	2.99	1.09-5.120	1.23	0.99-1.98
Leukemia	1.31	0.99-1.54	0.95	0.98-1.04	3.09	1.17-6.010	1.34	0.67-2.01
Joint and bone	1.45	1.09-1.98	1.00	1.00-1.06	2.48	1.32-4.790	2.12	1.76-3.01

OR: Odd ratios, 95% CI: 95% confidence intervals, [1]OR>1 means males are more susceptible than females to cancers

Table 3: ABO blood group distribution in cancer patients versus healthy controls

	Patients (1131)		Control (1200)				
Blood groups	N	(%)	N	(%)	UOR (95% CI)	AOR (95% CI)	p-value
O	358	31.70	405	33.75	1 (reference)	1 (reference)	0.531
A	445	39.35	397	33.08	1.51 (1.13-1.99)	1.41 (1.17-1.79)	0.014
AB	85	7.52	110	9.17	1.03 (0.97-1.41)	0,92 (0.67-1.21)	0.501
B	243	21.43	288	24.00	1.12 (1.08-1.57)	1.22 (1.17-1.65)	0.431

NS: Non significant, S: Significant, UOR: Univariate odd ratio, AOR: Adjusted odd ratio, multivariable logistic regression analysis was performed versus healthy control and adjusted for sex, age and type 2 diabetes

cancer patients and healthy controls was evaluated and the ORs were calculated to measure the association of each variable with different types of cancers, compared with healthy controls using multivariable logistic regression analysis.

Sex: The HCC, esophageal, pancreatic and gastric cancers were dominated by male patients, whereas female patients were at risk of the thyroid and breast cancer group. Therefore, thyroid and breast cancers showed a protective effect of male sex (OR = 0.46, 95% CI = 0.41-0.59) and (OR = 0.48, 95% CI = 0.44-0.61), respectively whereas all other cancers were positively associated with male sex compared with healthy controls.

Age: The age distribution of patients with gastrointestinal cancers (HCC, esophageal, gastric, biliary and pancreatic) was similar, whereas that of healthy controls and patients with other cancers (thyroid, breast, lymphoma and bone) had relatively younger age profiles. Thus, older individuals were more prone to cancer especially gastrointestinal tumors. All the other cancers analyzed here showed weak association with age. The age peak of cancer occurrence was different among various cancers. Thus, we calculated sex ratios within different age groups to avoid skewing by any particular age group. These sex ratios were found to be largely consistent among different age groups. Thus, the association of sex with different cancers seems to be independent of age.

Family history of cancer: The prevalence of a positive family history of cancer seemed to be higher in the cancer patients than controls. Also, there was stronger association the family cancer history with gastrointestinal cancer compared with other cancers.

Type 2 diabetes: The prevalence of type 2 diabetes was significantly higher in gastric, biliary and pancreatic cancers compared with other groups with OR ranging between 2.0 and 3.0. On the other hand, HCC and esophageal cancer were strongly inversely associated with type 2 diabetes compared with healthy controls (OR = 0.29, 95% CI = 0.15-0.55 and OR = 0.39, 95% CI = 0.24-0.65, respectively).

The distributions of blood types O, A, AB and B among cancer patients and controls were shown in Table 3. The highest frequent blood group seen among cancer patients was blood group A (39.35%), followed blood group O (31.7%), blood group B (21.43%) and blood group AB (7.52%). In control group, high frequency of blood group O (33.08%), followed by blood group A (33.75%), blood group B (24%) and blood group AB (9.17%). The distribution of blood type A was significantly higher among cancer patients than among healthy controls

Table 4: ABO blood group distribution frequencies among different types of cancers

		Groups							
		A n = 445		O n = 358		AB n = 85		B n = 243	
Cancer type	No. of patients	No.	(%)	No.	(%)	No.	(%)	No.	(%)
HCC	190	88	46.3	51	26.9	15	7.8	36	19.0
Esophageal	110	42	38.2	31	28.2	13	11.8	24	22.8
Gastric	125	39	31.2	59	47.2	8	6.4	19	15.2
Biliary	90	37	41.1	26	28.9	8	8.9	19	21.1
Pancreatic	95	58	61.1	10	10.5	6	6.3	21	22.1
Thyroid	85	30	35.3	34	40.0	5	5.9	16	18.8
Breast	111	44	39.6	34	30.6	14	12.6	19	17.1
Lymphoma	120	41	34.2	57	47.5	4	3.3	18	15.0
Leukemia	105	30	28.6	41	39.0	5	4.8	29	27.6
Skin	45	19	42.2	6	13.3	4	8.9	16	35.6
Joint, bone	55	17	30.9	9	16.4	3	5.5	26	47.3
Total patients	1131								

HCC: Hepatocellular carcinoma

(39.35% vs. 33.08%, p = 0.014), whereas, the distributions of blood type O, AB and B were similar between cases and controls (p>0.05). Compared with subjects with blood type O, the unadjusted odd ratio for the association of those with blood type A and cancer risk was 1.51 (1.13-1.99). Whether the association between the ABO blood group and cancer risk differed according to other known risk factors for cancer, including sex, age, family history and type 2 diabetes was also assessed. In multivariable logistic regression analysis, the adjusted OR was 1.41 (1.17-1.79) after adjusting for age, sex and type 2 diabetes.

The ABO blood group distribution frequencies in different types of cancers were shown in Table 4. Gastrointestinal tumors, especially HCC, constitute the majority of cancers in Dakahlia province in Egypt (54%) followed by lymph-proliferative malignancies and breast cancer. In HCC patients, a higher frequency of blood group A (46.3%), followed by group O (26.9%), B (19%) and AB (7.8%) was observed. In esophageal cancers, a higher incidence of blood group A (38.2%), followed by group O (28.2%), AB (22.8%) and B (11.8%) was observed. Amongst gastric cancers, a high frequency of blood group O (47.2%), followed by group A (31.2%), group B (15.2%) and AB (6.4%) was reported in this study. In Biliary cancer patients, a higher incidence of blood group A (41.1%), followed by group O (28.9%), B (21.1%) and AB (8.9%) was observed. Pancreatic cancer, a high frequency of blood group A (61.1%), followed by group B (22.1%), group O (10.5%) and AB (6.3%) was demonstrated. Thyroid cancer showed a high frequency of blood group O (40%), followed by group A (35.3%), group B (18.8%) and AB (5.9%). In breast cancers, a high incidence of blood group A (39.6%), followed by group O (30.6%), B (17.1%) and AB (12.6%) was seen. Lymphoma patients showed a preponderance of blood group O (47.5%), followed by group A (34.2%), B (15%) and AB (3.3%). Leukemia patients showed a high frequency of blood group O (39%), followed by group A (28.6%), B (27.6%) and AB (4.8%). In skin cancers, a high frequency of blood group A (42.2%)%), followed by group B (35.6%), group O(13.3%) and AB (9.8%) was reported a preponderance. Joint and bone tumor showed a high frequency of blood group B (47.3%), followed by group A (30.9.3%), group O (16.4%) and AB (5.5%). The incidence of blood group A was significantly higher in breast and HCC patients.

The association between ABO blood types and different cancer types were shown in Table 5. Blood group A was associated with significantly higher risk for malignancy including HCC

Table 5: Association between ABO blood types and cancer types

	A		B		AB		O	
Types	[1]OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
HCC								
Type O	1.55	1.12-2.16	0.98	0.69-1.41	0.80	0.63-1.08	-	
All other types	1.49	1.22-2.21	0.84	0.71-1.42	0.83	0.71-1.12	1.28	0.79-2.09
Esophageal								
Type O	0.99	0.71-1.40	1.54	1.20-1.99	1.25	0.77-2.12	-	
All other types	0.81	0.61-1.07	1.47	1.12-2.18	1.13	0.71-1.75	0.84	0.72-1.35
Gastric								
Type O	1.15	0.73-1.91	1.04	0.75-1.46	0.98	0.71-1.23	-	
All other types	1.25	0.89-1.96	1.02	0.77-1.37	0.92	0.75-1.24	1.27	0.79-1.78
Biliary								
Type O	0.92	0.71-1.21	1.49	1.12-2.16	0.65	0.55-1.07	-	
All other types	0.93	0.68-1.35	1.62	1.28-2.18	0.66	0.49-0.88	0.96	0.69-1.67
Pancreatic								
Type O	1.34	0.89-2.32	1.28	0.92-1.77	1.02	0.78-1.35	-	
All other types	1.29	0.81-1.97	1.21	0.91-1.65	0.89	0.70-1.04	1.09	0.79-1.47
Thyroid								
Type O	1.19	0.88-1.59	1.11	0.68-1.45	0.73	0.63-1.01	-	
All other types	1.29	1.02-1.87	1.20	0.85-1.71	0.76	0.61-0.99	1.25	0.78-1.87
Breast								
Type O	1.21	0.84-1.38	0.99	0.77-1.54	0.70	0.56-0.99	-	
All other types	1.31	1.11-1.66	0.98	0.84-1.34	0.75	0.55-0.91	1.08	0.73-1.58
Lymphoma								
Type O	1.31	1.01-1.71	1.13	0.72-1.74	0.82	0.62-1.05	-	
All other types	1.29	1.12-1.82	1.12	0.71-1.77	0.86	0.65-1.09	1.49	1.13-1.97
Skin								
Type O	1.19	0.99-1.47	1.07	0.75-1.57	0.63	0.59-1.08	-	
All other types	1.09	0.79-1.37	1.11	0.81-1.93	0.83	0.71-1.19	0.97	0.73-1.27
Leukemia								
Type O	1.27	0.89-1.54	1.01	0.66-1.49	0.72	0.58-0.99	-	
All other types	1.32	1.27-2.01	1.14	0.72-1.53	0.65	0.49-0.89	1.48	1.18-2.12
Joint, bone								
Type O	1.21	0.86-1.78	1.31	1.03-1.59	0.81	0.61-1.16	-	
All other types	1.25	0.88-1.85	1.42	1.12-1.99	0.88	0.65-1.19	0.96	0.71-1.28

OR: Odd ratios, 95% CI: 95% confidence intervals, For each cancer, every blood type group was compared with type O group using multivariable logistic regression analysis

(OR = 1.55, 95% CI = 1.12-2.16), Pancreatic (OR = 1.34, 95% CI = 0.89-2.32), skin (OR = 1.19, 95% CI = 0.99-1.47) and Breast cancers (OR = 1.21, 95% CI = 0.84-1.38) while biliary and esophageal cancer risk was significantly associated with blood type B (OR = 1.49, 95% CI = 1.12-2.16 and OR = 1.54, 95% CI = 1.20-1.99, respectively). The risk of thyroid and gastric cancers in blood type O were significantly higher when compared with the combination of all the other blood types (OR = 1.27, 95% CI = 0.79-1.78 and OR = 1.25, 95% CI = 0.78-1.87, respectively). In addition, Blood group O was associated with significantly higher risk for malignancy including lymphoma and leukemia (OR = 1.49, 95% CI = 1.13-1.97 and OR = 1.48, 95% CI = 1.18-2.12, respectively).

The comparison between level of Von Willebrand factor and anti-TF antibodies in patients and control group with blood group O were demonstrated in Table 6. In contrast to anti-TF antibodies

Table 6: Comparison between level of Von Willebrand factor and anti-TF antibodies in patients and control group with blood group O

Blood group O	Patients (371)	Control (457)		
Parameters	Mean±SD	Mean±SD	t	p
Von Willebrand factor IU m^{-1}	0.988±3.66	2.11±0.711	9.03	0.0001
Anti-TF antibodies IgM µg L^{-1}	22.4±7.1000	11.9±4.8000	5.70	0.0010

which were statistically significantly higher in cancer patients than healthy control, the von Willebrand Factor (VWF) was statistically significantly lower in cancer patients than healthy control ($p = 0.05$).

DISCUSION

In the current study, we have confirmed that blood group A is indeed associated with a higher risk of many cancers as, Hepatocellular Carcinoma (HCC), breast cancers, gastric cancer, pancreatic and skin cancers. In addition, we have further demonstrated that individuals with blood group O have a low risk of those cancers. A number of further needs considered when interpreting these results. Current result revealed that, HCC was considered the most common cancer in Dakahlia, Egypt. Poujol-Robert *et al.* (2006) explained the increased prevalence of blood group A among hepatocellular carcinoma by three reasons: Firstly, non-O blood group is an independent risk factor for the progression of liver fibrosis in HCV infection. Compared to those with blood type O, those with blood type A had a significantly more severely impaired liver function and an earlier onset of cirrhosis. These indicated an association between the ABO blood group and liver inflammation and fibrosis progression in patients with HCC. Secondly, a relationship between the ABO blood group and HCC is that the ABO blood group in conjunction with several important cytokines were known to be related to HCC development, including EGF, TNF-α, SICAM-1, E-selectin and P-selectin. Thirdly, the abnormal expression of ABO blood antigens in liver tissue might be another possible explanation for a relationship between the ABO blood group and HCC. ABO blood antigens (A, B, H) usually express on the surface of RBC and most epithelial tissue but not on hepatocytes, sinusoidal endothelial cells and bile canaliculi of the normal live. However, an increased ABH expression or neo-expression was observed in HCC tissues. The expression of the ABO blood group antigen was more severe in atypical adenomatous hyperplasia and hepatocellular carcinoma than in normal liver and chronic hepatitis. Okada *et al.* (1987) found neo-expression of ABH blood group antigens in HCC tissues. Expression of H-active glycolipid was enhanced in HCC tissues from the patients with blood types other than O. These suggest that alterations in glycosyltransferase specificity may occur during hepatic carcinogenesis. Recently, (Hoshida *et al.*, 2008) provided new insights into genome-based predictors of outcome in HCC patients. Also, our study revealed that the fourth most common cancer in Dakahlia, Egypt is breast cancer with high prevalence among blood group A. Our results were in agreement with those from (Anderson and Hass, 1984) who reported that, a study of rapidly progressive breast cancer in Tunisian women found a slightly increased risk of a positive diagnosis in blood type A. The increased rate of blood type A as compared to controls has been reported in breast cancer patients. The results suggest that the effect of blood type A on breast cancer development was capable of being masked by the effect of breast cancer susceptibility genes and/or that the inherited or non-inherited types involve different etiologic mechanisms. A high risk of early death in breast cancer patients with blood groups B and AB with AB having greater local recurrence risk has been reported. Sailaja *et al.* (2004) higher prevalence of blood group B in familial case of breast cancer than sporadic cases has been reported, supporting

familial breast cancer (Tryggvadottir *et al*., 1988) certain subtypes of breast cancer. Neither allele nor genotype frequencies differed significantly by age at diagnosis, tumor stage, grade and size, hormone, HER2 and lymph node status, intrinsic subtype or prognosis suggesting that the ABO blood group is not associated with either overall risk of or certain subtypes of breast cancer. In previous studies, contradictory reports are available about the association of blood group with breast cancer as increased B group or a group in breast cancer patients has been reported. No relation of breast cancer to any blood group (Jayant, 1971) increased B and O group (Tyagi *et al*., 1965) and increased B group (Surekha *et al*., 2004) in breast cancer patients has been reported previously.

High incidence of Gastric Carcinoma (GC) among those with blood group A (48%) was observed in current study. Quite similarly (Aird *et al*., 1953) has reported a strong association between GC and blood group A. The increased risk of development of gastrointestinal cancers in patients with blood group A has been explained by the expression of Forssman antigen in these cancers, which is structurally similar to the blood group antigen A. Because of this similarity, antibodies to A probably attack precancerous and cancerous cells expressing this antigen. Since people with blood group A lack antibodies to A, so they are more prone to develop these carcinomas. On the other hand, (Akhtar *et al*., 2011) have reported a higher frequency of blood group B was seen in gastrointestinal tract and gall bladder cancers, Quite similarly (Vioque and Walker, 1991) have reported a strong association of gall bladder and bile duct cancers with blood group B. Also, (Woo *et al*., 2013) reported that, ABO blood group antigens are widely distributed throughout the body in addition to their regular occurrence on the red blood cell surface. The ABO phenotype may be associated with risk of gastric cancer, gastric and duodenal ulcer, chronic atrophic gastritis as well as PC (Hussein, 2005; Tursen *et al*., 2005). Human PC has been shown to express either A or B antigens corresponding to the individual blood group or lose blood group antigen expression in 80% of the cases (Nakao *et al*., 2011). Deletion of A, B, H or Lewis antigens and incompatible expression of A or B antigens has been reported as a cancer-associated event in the pancreas. Incompatible expression of blood group related antigens is observed in PC cells, compared with patient blood group type, indicating that Lewis antigen expression in PC is independent of the blood group phenotype and may be useful as a tumor marker (Dandona *et al*., 2010).

A higher incidence of increased frequency of blood group B was seen in PC of recently published studies demonstrated the risk for PC of being significantly lower in patients with blood group O. The available evidence on the prognostic value of ABO blood group in patient with PC has been conflicting (Nakao *et al*., 2011; Wolpin *et al*., 2010). Thus, it could be hypothesized that genetic background associated with this blood group might predispose those patients who actually develop PC to a more favorable prognosis.

Several previously published studies investigated the possible ABO blood group correlation to cancer pancreas in many populations. Such correlations were demonstrated in Italian, Japanese, Turkish, Korean, Egyptian and North American patients. However, there is no conformity in all populations and these correlations are not consistent over all malignancies (Pelzer *et al*., 2013).

As regard skin cancer, several plausible explanations of the observed association with ABO blood groups. Certain biological mechanisms could associate ABO blood group with skin cancer. Blood group antigens expression on many epithelial cells surface, including skin cells may alter glycosyltransferase specificity influencing tumorigenesis. Another explanation is that ABO gene may be in linkage disequilibrium with other genes involved in skin tumorigenesis (Xie *et al*., 2010).

In this study, 131 cases of lymphoma was recorded with blood group A in 45 (34.4%), group O in 66 (50.4%), Group AB in 4 (3.1%) and group B in 16 (12.2%). Significantly higher incidence of lymphoma was observed in blood group O. No association of lymphoma with group O patient was reported earlier.

In this study, 84 patients had leukemia including AML and ALL. Leukaemia may yield weakened A or B antigen. In acute leukaemia, the A antigen may be weakened. Sometimes the blood appears to contain a mixture of group A and group O cells or of A1 and weak A. In other cases the red cells react weakly with anti-A, even behaving like A3. In a patient with erythroleukaemia of group B, 60% of the cells were not agglutinated by anti-B and appeared to be group O but were really very weak B, when separated from the normal B cells they would absorb anti-B (Gold *et al.*, 1959).

Moreover, a reported more favorable prognosis of patients with blood group O compared to patients with non-O blood group, whereas the survival between the individual blood groups did not differ significantly. Recent studies have suggested that SNPs at the ABO gene locus are associated with two serum markers of inflammation; Tumor Necrosis Factor-α (TNF-α) and soluble intercellular adhesion molecule-1 (SICAM-1) (Pare *et al.*, 2008). TNF-α is an inflammatory cytokine known to modulate the rate of pancreatic duct cell apoptosis. SICAM-1 inhibits lymphocyte attachment to endothelial cells and is significantly low in patients with blood group A or B compared to those with blood group O. Thus, decreased serum SICAM-1 levels in patients with non blood group O have been implicated in increased adhesion of leucocytes to endothelial surfaces promoting systemic inflammation and tumorigenesis (tumor development, progression and spread). Low plasma levels of SICAM-1 are also associated with increased risk of diabetes mellitus development, a known predisposing factor for PC. Collectively, we could postulate that ABO blood group possibly may correlate with the systemic inflammatory state, thereby influencing the risk of cancer (Mantovani *et al.*, 2008).

Our results revealed high level of anti-TF antibodies and Von Willebrand factor in cancer patients with blood group O than controls of same blood group and low. Kurtenkov *et al.* (1999) reported that blood group O also appears to exert a protective effect by preventing the growth and spread of tumors and being associated with longer survival times in cancer patients. Thomsen-Friedenreich antigen (TF), discovered in the late 1920s and the core disaccharide structure of ABO blood group (H) substance, is cryptic on cell membranes of various normal cells, including epithelial cells, Red Blood Cells (RBCs) and lymphocytes. During carcinogenesis, it appears with several other different tumor-associated glycol antigens, becomes immune-reactive and is expressed in many carcinomas, including those of the breast, colon, bladder and prostate (pancarcinoma marker) (Kurtenkov *et al.*, 2007).

TF has been postulated to have a role in adhesion and metastasis through tumor-endothelial cell interactions which is the key role in cancer metastasis and through binding ligands such as galectins or other lectins in sites of metastatic tumor growth, i.e., in the vascular endothelium, liver, bone marrow and lymph nodes. Due to antigenic similarity of TF to A antigen, blood group A individuals have the least aggressive humoral immune response against the TF than group O individuals, so it might be readily confused by the immune system of blood group A individuals (Kobayashi *et al.*, 2007).

Humans normally possess natural anti-TF antibodies (IgM) commonly induced in the gut, as many gram-negative organisms carry TF antigen. These bacteria grow more easily in blood group

O individuals and secretors than non-O individuals. Thus, they have high levels of natural anti-TF IgM and IgG antibody production and probably are less susceptible to cancer, have less aggressive disease and confer better prognosis. Studies have also shown that secretors have the highest natural anti-TF IgM level irrespective of ABO phenotype (Jenkins *et al*., 2006).

Experimentally, the passive transfer of anti-TF-Ag monoclonal antibody significantly extended the median survival time and inhibited lung metastasis in animals with metastatic 4T1 breast tumors. So, individuals with blood group O, secretors and post *E. coli* infection are strong responders of anti-TF antibody production, whether they are normal or cancer patients (Jaff, 2010).

Von Willebrand Factor (vWf) antigen has been demonstrated to be increased in patients with ovarian, bladder and colon cancers. It acts as an adhesive link between platelets and the endothelium correlating with more metastasis and poor prognosis. ABO genetics interact with secretor genetics influencing plasma levels of vWf with the highest vWf concentration in non-secretors and non-O blood groups and lowest concentration of vWf Ag in group O secretors (Morelli *et al*., 2007; Schleef *et al*., 2004).

Collectively, we could hypothesize that tumors have more chance to thrive and be more aggressive in patients with blood group A than those with blood group O. In addition to the less aggressiveness of tumors, blood group O might be associated with other diseases.

CONCLUSION

Blood group A apparently increases the risk for many cancers as breast cancer, HCC, gastric cancer, leukemia, thyroid, bone and joint and tumor of unknown origin than blood group O. Blood group O individuals have significantly higher incidence of secretor status than non-O blood group individuals. Therefore, it could be speculated that blood group O individuals with lower levels of vWf, higher natural anti-TF IgM and higher susceptibility to H. pylori and gram-negative intestinal flora infection are benefited and partially protected from certain malignancies or have less aggressive diseases.

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