Saudi Journal of Biological Sciences 29 (2022) 183–189

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

The association between two genetic polymorphisms in *ITGB3* and increase risk of venous thromboembolism in cancer patients in Eastern Province of Saudi Arabia

Asma Y. Alsulaim [a], Faisal Azam [b], Tunny Sebastian [c], Fathelrahman Mahdi Hassan [d], Sayed AbdulAzeez [e], J. Francis Borgio [e], Faisal M. Alzahrani [a,*]

[a] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[b] Consultant Medical Oncologist, King Fahad specialist Hospital, Dammam, Saudi Arabia
[c] Department of Clinical Nutrition, College of Applied Medical Sciences, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia
[d] Department of Hematology and Immunohematology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan
[e] Department of Genetic Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

ARTICLE INFO

Article history:
Received 1 June 2021
Revised 20 August 2021
Accepted 22 August 2021
Available online 27 August 2021

Keywords:
Genetic Polymorphisms
ITGB3 gene
Risk
Venous Thromboembolism
Cancer
Eastern Province
Saudi Arabia

ABSTRACT

Venous thromboembolism (VTE) is one of the major complications in most cancer patients leading to poor prognosis and short survival. Several common clinical risk factors coexist in cancer patients are used as risk predictive biomarkers to help in the management and prevention of VTE. These include cancer site and stage, chemotherapy regimen and elevated biological markers. However, Genetic polymorphisms in genes controlling coagulation and fibrinolysis **are** significantly associated with VTE if detected, then they might be more sensitive individual predictive biomarkers for VTE risk assessment. This study was conducted to evaluate the association between *ITGB3* rs3809865 and rs5918 with VTE risk as well as monitor the effect of VTE on overall survival of these cancer patients. In this retrospective case-control study, 195 cancer patients' formalin-fixed paraffin embedded tissue (FFPE) samples were collected (controls n = 157, case n = 38) using the stored data through Jan 2010 to Sep 2018 from King Fahad Specialist Hospital in Dammam. Samples were genotyped using TaqMan genotyping assay, then logistic regression analysis and Chi-square were used to predict the association between risk factors and VTE. Survival Comparison was tested by the log-rank test. Genetic polymorphisms in ITGB3 (rs3809865 and rs5918) found not to be associated with VTE increasing risk in cancer patients (p>0.05). While the advanced stage was potentially increasing the risk of VTE events (OR 5.1 CI 2.01–12.9p = 0.001). Patients with VTE showed a poor overall survival reflected by the median survival rate of only three years compared to seven years for cancer patients without VTE. This study highlighted the potential influence of VTE on prognosis and survival of cancer patients and raised the importance of exploring risk predictive biomarkers in our population. This will improve the risk prediction biomarkers leading to implementing safe and effective thrombosis prophylaxis strategies.

1. Introduction

Venous thromboembolism (VTE) is reported to be a relatively common complication in cancer patients (Donnellan and Khorana, 2017). Cancer increases the risk of venous thromboembolism by four to six folds leading to poor prognosis as well as increased rates of mortality, morbidity, and health care costs (Khalil et al., 2015, Eichinger, 2016, Angelini and Khorana, 2017; Hiraide et al., 2020). Recent study on the 499 092 patients revealed ninefold higher and increased risk of VTE in cancer patients compared to the general population (Mulder et al., 2021). Recent studies are suggesting development of specific recommendation for

* Corresponding author.
E-mail addresses: faisal.azam@kfsh.med.sa (F. Azam), tspurayidathil@iau.edu.sa (T. Sebastian), asayed@iau.edu.sa (S. AbdulAzeez), fbalexander@iau.edu.sa (J.F. Borgio), fmzahrani@iau.edu.sa (F.M. Alzahrani).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.08.073
1319-562X/

treating cancer types complicated with VTE (Zheng et al., 2021). It was estimated that 1.6–20% of cancer patients encounter VTE at any time during the disease course (Gran et al., 2018, Mohammed et al., 2015). Also, it was shown that VTE increases the rate of death in cancer patients by more than four folds as it is considered the second leading cause of death in this group of patients after cancer itself (Timp et al., 2013, Khalil et al., 2015, Donnellan and Khorana, 2017). These findings highlighted the importance of exploring potential factors that increase the risk of venous thromboembolism in cancer patients to manage and prevent VTE by establishing thrombosis prophylaxis plan (Iorga et al., 2019). Integrin Subunit Beta 3 (*ITGB3)* encodes for the beta subunit (IIIa) of the glycoprotein GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) (Database, 2019). This glycoprotein is considered the main receptor having a crucial role in platelet aggregation (Huang et al., 2019). Intracellular coagulation cascade events is activated upon the latest signalling process leading to irreversible aggregation and stable thrombus formation (Fullard, 2004). Current studies are directed towards identifying genetic markers specifically associated with an individual cancer patient to be used as individual risk factors predictors. Factor V Leiden mutation, prothrombin mutation (G20210A), methylenetetrahydrofolate reductase (MTHFR-A1298C), and (MTHFR-C677T) are studied extensively with conflicting results regarding the significant association **with VTE**. Most studies found that these polymorphisms increased the risk of venous thromboembolism in both cancer and non–cancer **patients** in the same proportion and did no show a significant difference in association in either group (Connolly and Francis, 2013). Conversely, a case-control study on breast cancer patients demonstrates a possible association between *FV* polymorphism that showed to be linked to increase Factor V expression and increase the coagulation activity when investigating polymorphisms in ***F5*** other than FV Leiden (Tinholt et al., 2014) . Interestingly, one of the recently studied genetic risk factors is *ITGB3* polymorphisms of the Glycoprotein IIIa (Bianconi et al., 2015). Three single nucleotide polymorphisms (SNPs) such as rs4642, rs5918 and rs3809865 in the *ITGB3* were studied for the association with VTE (Bianconi et al., 2015).The same previous study demonstrated a significant association between rs3809865 and VTE in colorectal cancer patients with ($p < 0.05$), while statistical analysis did not show any significant association between the other polymorphisms and the risk of VTE (Bianconi et al., 2015). Moreover, platelet bearing the glycoprotein IIIa with rs5918 polymorphism showed an increased risk for aggregation as it is assumed to have an increased affinity to fibrinogen. On the other hand, (Khatami et al., 2016, Xiang et al., 2016) reported contradictory conclusions in the association between rs5918 and venous thromboefmbolism. Allele frequency based on TOPMed (Trans-Omics for Precision Medicine) for SNPs rs3809865 and rs5918 are T = 0.304061 (80482/264690, TOPMED) and C = 0.123586 (32712/264690, TOPMED), respectively (Sherry et al., 2001). This research aim was to investigate the association between two SNPs rs3809865 and rs5918 in the *ITBG3* for the GP_{IIIa} of platelets with increased risk of venous thromboembolism in cancer patients in the Eastern Province of Saudi Arabia and to study the overall survival rate of cancer patients and to identify the median time of occurrence of VTE after the initial diagnosis of cancer.

2. Materials and methods

A retrospective case-control study was conducted in Imam Abdulrahman bin Faisal University in accordance with the Declaration of Helsinki. This research protocol was approved by Imam Abdulrahman bin Faisal University Institutional review board (IRB-PGS-2018–03-136) and King Fahad Specialist Hospital (LAB0311). The study samples for this research were archived formalin-fixed paraffin embedded tissue (FFPE) samples. For that, waiver consent was sufficient for approval of the study sample collection. The study population for this research was 195 cancer patients diagnosed at King Fahad Specialist Hospital in Dammam (KFSHD). The inclusion criteria for controls were as follow, Saudi and non-Saudi of both gender patients older than 18 years with a confirmed diagnosis of cancer by histopathological studies. Additional inclusion criteria for cases were venous thromboembolism VTE which was confirmed by radiological studies. However, the exclusion criteria for both cases and controls were paediatric cancer patients, patients with haematological malignancies and cancer patients with known FV Leiden, prothrombin, *MTHFR* mutation carriers.

Cancer patients confirmed using histopathology were proven VTE using routine diagnosis. Radiological reports of cancer patients from Jan 2010 to Sep 2018 were collected using ultrasound venous doppler as code for VTE cases that fit the inclusion criteria of the study. A total of 45 cases were available and out of them only 38 (Breast cancer 24; Colon cancer 7; Ovarian cancer 1; Brain cancer 3; Lung cancer 2; and Bladder cancer 1) were included in the research for one of these reasons either the tissue blocks were used for another research or only one block was available for the case and using these blocks for research is against IRB rules. Simple random sampling technique that was done using IBM SPSS Statistics 21 software (Hong Kong, China) was used to select 157 (Breast cancer 76; Colon cancer 41; Ovarian cancer 8; Brain cancer 10; Lung cancer 6; and Bladder cancer 16) control samples. Demographic data and clinical history were collected for selected cases. Data collected include age, gender, date of initial cancer diagnosis, site and stage of cancer, date of VTE occurrence, history of hospitalization, chemotherapy regimen used and date of death.

Tissue blocks containing soft tissue, medium-sized, low fat content and perfect tissue embedding were accepted. From each selected FFPE tissue, four sections with 10 μm thickness were cut using the Rotary Microtome (LEICA RM 2255 Rotary Microtome by Leica Biosystems (IL, USA) then placed in a 1.5 ml sterile microcentrifuge tube. Melting the paraffin using hazard solvents such as ethanol and xylene described by was performed in this research with minor modifications (Sikora et al., 2011). Silica membrane-based DNA extraction was the method of choice provided by DNeasy Blood and Tissue extraction kit (Qiagen, Hilden, Germany). DNA concentration and purity were measured by Nanodrop 8000 spectrophotometer by Thermo Scientific (DE, USA) and samples were stored at −80 °C to be used for further analysis.

2.1. Genotyping of the studied variants

TaqMan based SNP Genotyping assay was conducted by 7500 Fast Real-Time PCR System (Applied Biosystems™). The flanking sequences of SNP rs3809865 in *ITGB3* was obtained from the national centre for biotechnology information (NCBI) with sequence reference (GenBank accession number: NC_0000171.11). Forward and reverse primers flanking the SNP of interest were selected to have an amplicon length between 700 and 800 bp with a C/G content between 40 and 60 %. Complete primers design was achieved by NCBI primer blast together with reverse complement tools. Amplification was optimized using primers, As9865F [(Forward) 5′ ATGGTTCTCTCGCAAGG 3′] and As9865R [(Reverse) 5′ GTGACTTGCTGTGAATGAT 5′] through gradient PCR. Agarose gel electrophoresis was used for DNA fragments separation according to the fragment size. Agarose gel was then transferred to the Molecular Imager® ChemiDoc Doc™ XR System, Bio-Rad (CA, USA) to visualize DNA under UV light. PCR products were purified from impurities like as primers, nucleotides, enzymes, and salts using QIAquick®purification kit. Amplicons

were sequenced using 3500 genetic analyser (Applied Biosystems™ HITACHI, Austin, USA) through Sanger sequencing technology. Purification of PCR **products** after cycle sequencing was done to remove unbound BigDye® terminators and salts. This step was archived by Big dye terminator™ purification kit, Applied biosystem. The sequencing analysis software mutation surveyor® software was used for *ITGB3* sequencing analysis.

IBM SPSS v.21 software was used in this study for descriptive statistical analysis. Mean and standard deviation as well as median and interquartile ranges were used to present the continuous variables. Nominal variables frequencies were presented as percent and numbers. Chi square and logistic regression test were used to compare the frequencies between cases and controls clinical history. It was also used to assess the association between rs3809865 and rs5918 and VTE. Kaplan-Meier Curve and log rank test were used to compare survival time and asses its significance between the study groups. A p-value < 0.05 within 95% confidence interval was considered statistically significant Hardy Weinberg equilibrium and minor allele frequency were calculated using Haploview 4.2 software (MA, USA).

3. Results

Study population detailed demographics characteristics and clinical data were presented in Table 1, they were divided into two groups: case (n = 38) and controls (n = 157). The mean age of the study population in both cases and controls was 58 years with a mean BMI for cases and controls of approximately 29.2 and 28.1, respectively. The predominance of female patients was demonstrated in both case and control groups accounting for 72.3% of the total study population. Hospitalization status which included any hospital stay more than 4 days as well as patients under palliative care presented 69% of the study population with no significant difference between cases and controls. More than 70% of cases were dead compared to only 17% in controls. In this study, two cancer treatments data known to be a risk factor of VTE were collected which included chemotherapy and hormonal therapy. Most cases were under chemotherapy presenting 92.1%, while for hormonal therapy only 44.7% were under this type of treatment. Advanced stage cancer patients were higher in cases compared to controls accounting for 84%, 50%, respectively. Chemotherapy as well as advanced stage of cancer showed a statistically significant *p*-value of 0.04 and 0.01, respectively by univariate analysis. But, with logistic regression chemotherapy showed statistically insignificant *p*-value of 0.56 while advanced stage showed *p*-value of 0.001(OR 5.1 CI 2.0–12.9) which indicates advanced stage significant association with VTE occurrence. The samples used for this research were subjected to TaqMan genotyping analysis without any exclusion. As known that the effect of DNA quality will be demonstrated by the success or failure in the final analysis. Only three samples were excluded from the research as they were below the accepted DNA concentration. A total of 192 samples were genotyped using the TaqMan® Genotyping assay. Genotypes were then analyzed by the TaqMan software. The software fluorescence calls were automatically plotted on the allelic discrimination plot which discriminates each allele as a specific cluster. The allelic discrimination plot was divided into three clusters. Lower right cluster (red) represents homozygous for allele 1, upper left cluster (blue) represents homozygous for allele 2, while the center cluster represents heterozygous for allele 1 / allele 2 Allele specifications were determined by rs3809865A (VIC™) / rs3809865T (FAM™) and rs5918C (VIC™) / rs5918T (FAM™).

Allele distribution for the study SNPs differs between cases and controls. For rs3809865, higher frequency of heterozygous genotype AT was demonstrated in the cases showing 51.4% compared to 36.4% in controls. On the other hand, homozygous genotype AA showed a higher frequency in controls compared to only 31.4% in cases. However, these differences in frequency were statistically insignificant by the bivariate analysis showing a *p*-value of 0.31 for genotype AA and a *p*-value of 0.98 for genotype AT. Homozygous genotype TT showed the least frequency in cases and controls being 17% and 11%, respectively. For rs5918, homozygous TT genotype showed the highest frequency, 69.7% and 73% for cases and controls, respectively. Heterozygous genotype CT was slightly higher in controls being 22% compared to 27% in case. Lowest frequency was the carriers of homozygous genotype CC. The bivariate analysis reflected statistically insignificant differences between the cases and controls represented by a *p*-value of 0.53 and 0.64 for genotypes CT and CC, respectively. Genotypes frequency distributions are shown in Table 2. The minor allele for both SNPs was (T). Minor allele frequency (T) of rs3809865 was found to be 0.317, while for rs5918 was 0.162 in the study population (Table 3). Hardy Weinberg equilibrium was calculated for these SNPs by comparing the differences between predicted and observed heterozygosity of these SNPs and found to obey the Hardy Weinberg equilibrium (HWE). Three known samples were sequenced to be used as positive quality control samples for the SNP rs3809865. Sequenced samples analyzed by mutation sur-

Table 1
Characteristics and clinical data of the study population.

Variable	Case (n %)	Control (n %)	Total (n %)	p-value** (χ^2)
Age (year)				
Mean	58 ± 12.4	58 ± 13.1		
BMI				
Mean	29.2 ± 10	28.1 ± 6		
Gender				
Female	31 (81.6%)	110 (70.1%)	141(72.3%)	0.15
Male	7 (18.4%)	47 (29.9%)	54 (27.7%)	
Cancer treatment				
Chemotherapy	35 (92.1%)	122 (77.7%)	157 (80.5%)	0.04
Hormonal therapy	17 (44.7%)	47 (29.9%)	64 (32.8%)	0.08
Stage				
Local	6 (16.2%)	78 (49.7%)	84 (43.3%)	0.01
Advanced / metastasis	31 (83.8%)	79 (50.3%)	110 (56.7%)	
Hospitalization	29 (76.3%)	103 (67.3%)	132 (69.1%)	0.2
Mortality				
Dead	27 (71.1%)	27 (17.4%)	54 (28.0%)	<0.001
Alive	11 (28.9%)	128 (82.6%)	139 (72.0%)	

** p < 0.05 is statistically significant.

Table 2
Genotype and frequency distribution.

SNP ID	Genotypes	Genotype Frequencies		p-value** (χ^2)
		Case (%)	Control (%)	
rs3809865	AA	11 (31.4%)	81 (52.6%)	0.310
	AT	18 (51.4%)	56 (36.4%)	0.980
	TT	6 (17.1%)	17 (11%)	Reference*
rs5918	CT	9 (27.3)	33 (21.7%)	0.53
	CC	1 (3 %)	8 (5.3 %)	0.64
	TT	23 (69.7%)	111 (73%)	Reference*

* The reference genotypes used the chi-square (χ^2) to compare genotypes frequencies;
** $p < 0.05$ is statistically significant.

Table 3
Minor allele frequencies and HW p-value.

SNP	Minor Allele	MAF	Predicted heterozygosity	Observed Heterozygosity	HW p-value *
rs3809865	T	0.317	0.433	0.392	0.232
rs5918	C	0.162	0.272	0.272	0.054

* $p < 0.05$ Obeying Hardy Weinberg equilibrium; MAF: Minor allele frequency.

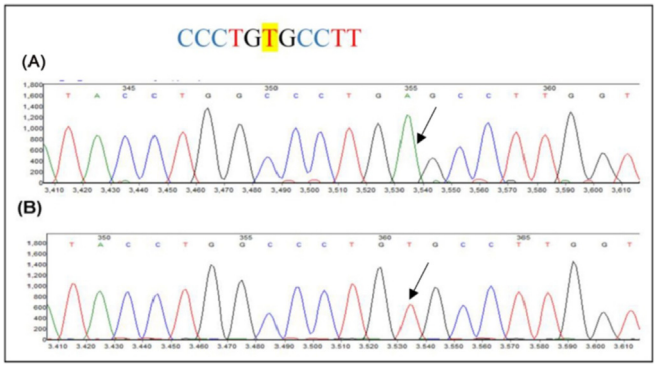

Fig. 1. Representative of direct sequence electropherogram of the gene region, ITGB3. Arrow indicates the position of rs3809865. (A) Sequence with genotype AA of rs3809865. (B) Sequence with genotype TT of rs3809865. A clear peak in electropherogram indicates a homozygous result.

veyor® software results reflected the specific genotype result from the TaqMan® genotyping assay. The sequence results are shown with an example of a sequence chromatogram in Fig. 1. To investigate the association between rs3809865 and rs5918 with VTE, binary logistic regression analysis was used for this investigation. The Genotype TT was used as the reference for both SNPs according to the NCBI reference sequence. Crude logistic regression analysis of rs3809865 showed an odds ratio of 2.6 (95% CI 0.845–7.99) for genotype AA with a *p*-value pf 0.096, reflecting a statistically insignificant association between VTE and genotype AA in the study population. Genotype AT for the same SNP also did not show any significant association with a *p*-value of 0.86 and odds ratio of 1. Investigating the association between rs5918 and VTE reveals a lack of association of both CC and CT genotypes which was reflected by statistically insignificant of *p*-value for both genotypes. Genotype CC regression analysis resulted in odds ratio 1.6 (95% CI 0.198–13.9, *p*-value 0.64) and for genotype CT the odds ratio was 0.76 (95% CI 0.321–1.8, *p*-value 0.53). Table 4 presents crude regression analysis data details for both SNPs.

The duration between the initial diagnosis of cancer and VTE occurrence for the cases was estimated by the calculated median and interquartile range. The median of VTE occurrence was about 22 months after the initial diagnosis of cancer with 25% of cases who experience VTE within five months or less since the initial diagnosis of the cancer. On the other hand, 25% of cases were diagnosed with VTE after 51 months or more since the first cancer diagnosis. Survival probabilities between cases and controls were examined by Kaplan Meier survival plots. Two samples were excluded from this analysis due to the unknown death date. As demonstrated in Kaplan Meier survival plot of Fig. 2 the probabilities of cases survival were low compared to survival probability in controls which clearly indicates a poor survival of cases. A 2-year survival probability in cases was approximately 64% compared to higher survival in controls which was 90%. The log rank (Mental -Cox) test was also used to explore this difference in survival which was statistically significant with *p*-value < 0.001. Overall mean survival was around three years for cases and seven years for controls.

4. Discussion

Cancer patients encounter multiple complications during the disease course. One of the most common complications is VTE

Table 4
Crude binary logistic regression for rs3809865 and rs5918 using the VTE occurrence as the dependent variable.

SNP ID	Genotype	p-value	Odds ratio	95% C.I**	
rs3809865	AA	0.096	2.6	0.845	7.995
	AT	0.86	1	0.376	3.206
	TT	Reference*	–		
rs5918	CT	0.53	0.760	0.321	1.801
	CC	0.64	1.658	0.198	13.904
	TT	Reference*	–		

* Reference genotypes used for regression analysis; $p < 0.05$ is statistically significant.
** CI: Confidence Interval,

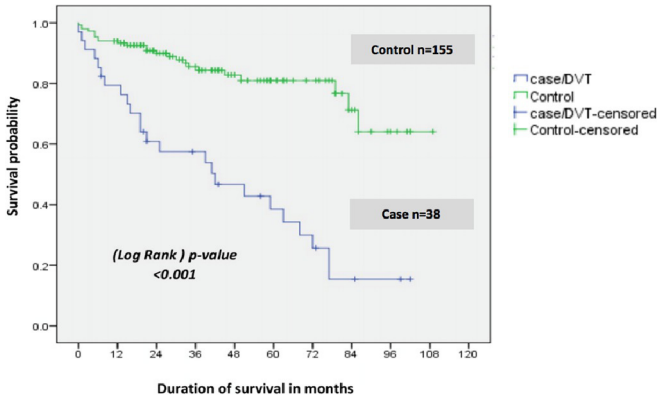

Fig. 2. Kaplan Meier survival curve. Kaplan Meier survival comparison between cases and controls shows clear difference in survival probabilities. Log Rank survival analysis revealed a statistically significant difference between cases and control survival with a p-value < 0.001.

which correlates with increased mortality, morbidity in these patients. Identifying risk factors correlated to VTE occurrence will help to constrain its significant effects by selecting the VTE high-risk cancer patients. Cancer patient categorization depending on risk are based on different VTE prediction assessment tools. Thrombosis risk may arise from cancer itself as the primary cancer site, stage and grade of cancer. Surgery, chemotherapy, hormonal therapy, antiangiogenic drugs and the central catheter can raise the risk of thrombosis in relation to different treatment modalities described for cancer patients. Lastly, multiple clinical parameters if encountered by the patient, will increase the risk of VTE events, like, abnormal white blood cell count, platelet count, d-dimer and P-selection (Khalil et al., 2015, Fernandes et al., 2019). Recently, studies are concentrating on exploring genetic risk factors related to thrombosis risk, specifically in cancer patients. It is hypothesized that these genetic factors may add precision to the risk assessment tools (Muñoz Martín et al., 2018). Our retrospective case-control hospital-based study mainly aimed to investigate the association between rs3809865, rs5918 SNPs in *ITGB3* and VTE occurrence increase risk in cancer patients. Additionally, exploring the survival rate of these patients and observing the time of VTE occurrence during cancer disease. The major findings of this study were the lack of association between rs3809865, rs5918 in *ITGB3* with an increased risk of VTE in cancer patients after the adjustment of covariates. The cancer stage showed a significant effect on increasing VTE risk. Monitoring the VTE events time occurrence and survival rate of cancer patients with VTE revealed that 25% of the cases had a VTE event in the first year after cancer diagnosis initially and all cases had poor survival which was directly linked to the VTE occurrence. These findings affirm the impact of venous thrombosis on cancer patients' overall survival. The frequency of rs3809865 genotypes between cases and controls was slightly different but not reaching the statistical significance reflecting the lack of association with increased risk. Correspondingly, Bianconi et al., 2015 reported an eight-fold increase in the risk of developing venous thromboembolism in colorectal cancer patients carrying AA genotype of rs3809865. As mentioned, the later research enrolled only colorectal cancer patients. While in our study, study population cancer types were not specified, and this may explain the conflict results between the two studies as this polymorphism may be specific only to colorectal cancer groups. No studies were found examining the effect of this SNP on platelet function. For the second SNP in this study, we investigated the association between rs5918 with increasing VTE risk and the results confirmed the absence of any association. The prospective study that was done by Bianconi et al., 2015 revealed that the presence of rs5918 in cancer patients does not carry any risk for VTE occurrence. Similarly, (Xiang et al., 2016) had explored 29 variants in the *ITGB3*, including rs5918 in the Chinese population, to analyse their influence on GPIIb/IIIa receptor expression and their impact on platelet function. The study demonstrated no effect on platelet function and thus no association with thrombosis. Likewise, (Bennett et al., 2001) showed no impact of this SNP on platelet function. However, Komsa-Penkova et al., 2017 explored if rs5918 polymorphism carriers exhibit higher risk for developing VTE. They found that it significantly contributes to increased risk of VTE in the general population only as no cancer patients were included in their study. Also, they noted the increased risk in relation to this polymorphism to be higher in females compared to males. Cancer patients with metastasis or advanced-stage cancer comprising 84% of cases showed an increased risk of VTE in our study. Similarly, Abdol Razak et al., 2018 reported that the risk of VTE in cancer patients with metastasis could reach 58-fold increased risk compared to patients with local cancer.(Khalil et al., 2015) also considered the cancer patients with distant metastasis as VTE high-risk group. Conversely, Khorana and Connolly, 2009, indicated that the stage of cancer was not predictive of VTE risk occurrence in the case of ambulatory cancer patients. Chemotherapy carries an adverse effect on the cancer patients by causing endothelial damage initiating an abnormal thrombosis response which may cause VTE Maia et al., 2019). In our study, chemotherapy had no impact on increasing VTE risk. Other studies declared that the occurrence of VTE is directly connected to the initiation of chemotherapy (Chen et al., 2018). This finding was also reported by Maia et al., 2019, who found that the risk of VTE in lung cancer patients increases after six months following the chemotherapy initiation. Wang et al., 2019 similarly documented a significant association of chemotherapy with VTE as well. Moreover, (Abdol Razak et al., 2018) stated that the VTE risk associated with chemotherapy differs according to cancer type; highlighting stomach and pancreatic cancer to carry a high VTE risk compared to other cancer types. (Khorana and Connolly, 2009) explored the risk of the specific combination of chemotherapy drugs with increasing VTE events and noted that each chemotherapy drug carries a different risk of causing VTE in cancer patients. As demonstrated above, our results did not match the literature findings, and this may arise from the retrospective nature of the study or different chemotherapy regimens followed in our hospital that may not have any influence on the thrombosis system. Venous thromboembolism risk is distinct in each type of cancer depending on specific cancer mechanisms causing thrombosis (Abdol Razak et al., 2018). In our study, cancer types were not defined as a separate VTE risk factor due to the limited cases of VTE in our study population. Aleem et al., 2012 revealed that breast, lung cancer and non–Hodgkin's lymphoma was the most common types showed high VTE risk. While Abdol Razak et al., 2018 stated that lung, brain, pancreas and kidney carry the highest risk. Furthermore, Sheth et al., 2017 demonstrated that brain, pancreas and stomach were the highest at risk. As noted, that reports indicating the common primary site of cancer with the highest risk were controversial, this may be influenced by the frequency of each cancer in the population studied. In this study, we compared the survival of cancer patients who encounter VTE with cancer patients without VTE (controls). The results revealed 79% of cases with one-year survival of compared to a 94% for controls. Similarly, a two-year survival was lower by more than 10% in cases but still around 90% in controls. As known that survival may be affected by many factors besides the VTE itself. In our study, the mortality was significantly associated with VTE according to multivariate analysis after covariates adjustments. Also, Mohammed et al., 2015 noted a one-year survival of cases to be only 18.9% with a median survival of 12.4 months in a retrospective study conducted at King Abdullah

Medical city in the western region. Similarly, A recently published retrospective study by Maia et al., 2019 on lung cancer patients confirmed a poor prognosis and reduced overall survival related to VTE occurrence, which was 29.1% survival rate with a median survival of 1.5 months. Conversely, in a study comprising pancreatic cancer patients with advanced-stage, Chen et al., 2018 reported no differences in survival between cancer patients with VTE and without VTE, however, they observed a poor survival in patients with early VTE events with a median survival of 1.8 months compared to late VTE. Chen et al., 2018 included only pancreatic cancer patients with advanced-stage and this may explain the insignificant difference in survival. Also, they confirmed the association of advanced-stage cancer with an increased risk of VTE which was found in our research as well. The discrepancies between these studies may be influenced by sample size, study design, generalized vs. specific cancer sites which highlight the need for further research. Comorbidities like cancer disease progression, renal disease, pulmonary disease, infections, and other factors that may add risk to poor survival should alsobeconsidered when monitoring overall survival. As observed by Chen et al., 2018, the early-onset venous thromboembolism was linked with poor survival. This observation highlighted the importance of VTE screening since the initial diagnosis of cancer as the risk increase in the first months after diagnosis. In this study, 25% of cases encountered VTE in the first six months while the remaining had VTE after one year with a median duration of 22 months from cancer diagnosis to VTE occurrence. On the other hand, it was observed by Agnelli et al., 2014 who monitored the VTE risk prospectively, that the risk was 2–folds higher in the first 12 months after an initial cancer diagnosis. Similarly, a study published by Wang et al., 2019 found that the VTE cases increased significantly in the first three months affecting 50% of cases and the risk of death increases after three months of VTE event.

A recent systematic review published by Mulder et al., 2019, analysing the most validated risk assessment tools named Khorana risk assessment tool for ambulatory cancer patients, revealed that most VTE events occurred within the low and intermediate-risk groups. This observation reflects that more studies are required to validate more predictive risk factors to establish precise assessment tools. In King Faisal Specialist Hospital in Riyadh, the Caprine VTE assessment tool is now followed as a mandatory measure for all hospitalized patients before admission considering cancer patients as a very high-risk group. In ambulatory cancer patients the Khorana risk assessment tool is used but as an optional measure to be decided according to the case of the patients by the physician. The retrospective design of the study was a significant limitation causing the enrolment of only a small sample size of the VTE cases due to the difficulty of radiological reports retrieval from the system. The chance of a repetition of any failure of the extraction procedure was limited because of the FFPE samples used in the study. Also, cancer cases included in this study without stratification of the cancer types making the study generalized rather than cancer specific. To our knowledge, this is the first study in Saudi Arabia to investigate genetic predictive risk factors related to VTE risk in cancer patients.

5. Conclusion

Venous thromboembolism showed a major complication of cancer in our study population. The molecular and clinical predication biomarkers for VTE risk in Saudi Arabian population are limited and need further research. This step is beneficial to establish the appropriate thromboprophylaxis plans maximizing the efficacy of anticoagulation treatments and minimizing the risk of bleeding. We recommend running this study in a prospective design to monitor the cancer patients closely and collect the required data precisely as needed. Larger study population with a case: control ratio with 1:1 or 1:2 for an accurate statistical comparison is recommended too. Also, it is recommended to conduct this study on a specific cancer type starting from the common type in the region. High throughput molecular technologies are recommended to explore the VTE molecular prediction markers. Finally, exploring the clinical VTE risk factors in our population is highly recommended.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Special thanks to all research assistants (Mr. Ranilo. M. Tumbaga, Mr. Horace T. Pacifico, and Mrs. Jee Entusiasamo Aquino) at the Department of Genetic Research, Institute for Research and Medical Consultations, Heath informatics department (Mr. Essam Elmemegy), Histopathology laboratory (Mohammed Foqha) and Radiology Department (Dr. Najla altuwaijri) at king Fahad specialist hospital Dammam who helped us during the conduction of this study, Also thanks to the Deanship of Scientific Research, Imam Abdulrahman Bin Faisal University for supporting this work.

References

Abdol Razak, N.B., Jones, G., Bhandari, M., Berndt, M.C., Metharom, P., 2018. Cancer-associated thrombosis: an overview of mechanisms, risk factors, and treatment. Cancers 10 (10), 380.

Agnelli, G., Verso, M., Mandalà, M., Gallus, S., Cimminiello, C., Apolone, G., Di Minno, G., Maiello, E., Prandoni, P., Santoro, A., Crinò, L., 2014. A prospective study on survival in cancer patients with and without venous thromboembolism. Internal and emergency medicine 9 (5), 559–567.

Aleem, A., Diab, A.R.A., Alsaleh, K., Algahtani, F., Alsaeed, E., Iqbal, Z., El-Sherkawy, M.S., 2012. Frequency, clinical pattern and outcome of thrombosis in cancer patients in Saudi Arabia. Asian Pacific Journal of Cancer Prevention 13 (4), 1311–1315.

Angelini, D. and Khorana, A.A., 2017, July. Risk assessment scores for cancer-associated venous thromboembolic disease. In Seminars in thrombosis and hemostasis (Vol. 43, No. 05, pp. 469-478). Thieme Medical Publishers.

Bennett, J.S., Catella-Lawson, F., Rut, A.R., Vilaire, G., Qi, W., Kapoor, S.C., Murphy, S., FitzGerald, G.A., 2001. Effect of the PlA2 alloantigen on the function of β3-integrins in platelets. Blood, The Journal of the American Society of Hematology 97 (10), 3093–3099.

Bianconi, D., Schuler, A., Pausz, C., Geroldinger, A., Kaider, A., Lenz, H.-J., Kornek, G., Scheithauer, W., Zielinski, C.C., Pabinger, I., Ay, C., Prager, G.W., 2015. Integrin beta-3 genetic variants and risk of venous thromboembolism in colorectal cancer patients. Thrombosis research 136 (5), 865–869.

Chen, J.S., Hung, C.Y., Chang, H., Liu, C.T., Chen, Y.Y., Lu, C.H., Chang, P.H., Hung, Y.S., Chou, W.C., 2018. Venous thromboembolism in asian patients with pancreatic cancer following palliative chemotherapy: Low incidence but a negative prognosticator for those with early onset. Cancers 10 (12), 501.

Connolly, G.C. and Francis, C.W., 2013. Cancer-associated thrombosis. Hematology 2013, the American Society of Hematology Education Program Book, 2013(1), pp.684-691.

Database, G. H. G. 2019. ITGB3 Gene - GeneCards | ITB3 Protein | ITB3 Antibody.

Donnellan, E., Khorana, A.A., 2017. Cancer and venous thromboembolic disease: a review. The Oncologist 22 (2), 199–207.

Eichinger, S., 2016. Cancer associated thrombosis: risk factors and outcomes. Thromb Res 140 (Suppl 1), S12–S17.

Fernandes, C.J., Morinaga, L.T.K., Alves, J.L., Castro, M.A., Calderaro, D., Jardim, C.V.P., Souza, R., 2019. Cancer-associated thrombosis: the when, how and why. Eur Respir Rev 28.

Fullard, J.F., 2004. The role of the platelet glycoprotein IIb/IIIa in thrombosis and haemostasis. Curr Pharm Des 10, 1567–1576.

Gran, O.V., Brækkan, S.K., Hansen, J.B., 2018. Prothrombotic genotypes and risk of venous thromboembolism in cancer. Thromb Res 164 (Suppl 1), S12–S18.

Hiraide, M., Shiga, T., Minowa, Y., Nakano, Y., Yoshioka, H., Suzuki, K., Yasuda, C., Takahashi, H., Hama, T., 2020. Identification of risk factors for venous thromboembolism and evaluation of Khorana venous thromboembolism risk assessment in Japanese lung cancer patients. J Cardiol 75 (1), 110–114.

Huang, J., Li, X., Shi, X., Zhu, M., Wang, J., Huang, S., Huang, X., Wang, H., Li, L., Deng, H., Zhou, Y., Mao, J., Long, Z., Ma, Z., Ye, W., Pan, J., Xi, X., Jin, J., 2019. Platelet integrin αIIbβ3: signal transduction, regulation, and its therapeutic targeting. J Hematol Oncol 12, 26.

Iorga, R.A., Bratu, O.G., Marcu, R.D., Constantin, T., Mischianu, D.L.D., Socea, B., Gaman, M.A., Diaconu, C.C., 2019. Venous thromboembolism in cancer patients: Still looking for answers. Exp Ther Med 18, 5026–5032.

Khalil, J., Bensaid, B., Elkacemi, H., Afif, M., Bensaid, Y., Kebdani, T., Benjaafar, N., 2015. Venous thromboembolism in cancer patients: an underestimated major health problem. World J Surg Oncol 13, 204.

Khatami, M., Heidari, M.M., Soheilyfar, S., 2016. Common rs5918 (PlA1/A2) polymorphism in the. Arch Med Sci Atheroscler Dis 1, e9–e15.

Khorana, A.A., Connolly, G.C., 2009. Assessing risk of venous thromboembolism in the patient with cancer. J Clin Oncol 27, 4839–4847.

Komsa-Penkova, R., Golemanov, G., Tsankov, B., Ivanov, P., Beshev, L., Tonchev, P., 2017. Rs5918ITGB3 Polymorphism, Smoking, and BMI as Risk Factors for Early Onset and Recurrence of DVT in Young Women. Clin Appl Thromb Hemost 23, 585–595.

Mohammed, A.A., Al-Zahrani, A.S., Farooq, M.U., Ghanem, H.M., Abdelaziz, L.A., El-Khatib, H.M., 2015. Pattern of Venous Thrombosis in Cancer Patients: Frequency and Survival Effect; Single Center Experience. Indian J Hematol Blood Transfus 31, 439–445.

Mulder, F.I., Candeloro, M., Kamphuisen, P.W., Di Nisio, M., Bossuyt, P.M., Guman, N., Smit, K., Büller, H.R., Van Es, N., Collaborators, C.-P., 2019. The Khorana score for prediction of venous thromboembolism in cancer patients: a systematic review and meta-analysis. Haematologica 104, 1277–1287.

Mulder, F.I., Horvàth-Puhó, E., van Es, N., van Laarhoven, H.W.M., Pedersen, L., Moik, F., Ay, C., Büller, H.R., Sørensen, H.T., 2021. Venous thromboembolism in cancer patients: a population-based cohort study. Blood 137 (14), 1959–1969.

Muñoz Martín, A.J., Ortega, I., Font, C., Pachón, V., Castellón, V., Martínez-Marín, V., Salgado, M., Martínez, E., Calzas, J., Rupérez, A., Souto, J.C., Martín, M., Salas, E., Soria, J.M., 2018. Multivariable clinical-genetic risk model for predicting venous thromboembolic events in patients with cancer. Br J Cancer 118, 1056–1061.

Maia, R., Neves, I., Morais, A., Queiroga, H., 2019. Venous and lung thromboembolism in the context of lung cancer: clinical manifestations, risk factors and prognosis. Acta medica portuguesa 32 (10), 647–653.

Sheth, R.A., Niekamp, A., Quencer, K.B., Shamoun, F., Knuttinen, M.G., Naidu, S., Oklu, R., 2017. Thrombosis in cancer patients: etiology, incidence, and management. Cardiovasc Diagn Ther 7, S178–S185.

Sherry, S.T., Ward, M.H., Kholodov, M., Baker, J., Phan, L., Smigielski, E.M., Sirotkin, K., 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Research 29 (1), 308–311.

Sikora, M.J., Thibert, J.N., Salter, J., Dowsett, M., Johnson, M.D., Rae, J.M., 2011. High-efficiency genotype analysis from formalin-fixed, paraffin-embedded tumor tissues. Pharmacogenomics J 11, 348–358.

Timp, J.F., Braekkan, S.K., Versteeg, H.H., Cannegieter, S.C., 2013. Epidemiology of cancer-associated venous thrombosis. Blood 122, 1712–1723.

Tinholt, M., Viken, M.K., Dahm, A.E., Vollan, H.K., Sahlberg, K.K., Garred, O., Børresen-Dale, A.L., Jacobsen, A.F., Kristensen, V., Bukholm, I., Kåresen, R., Schlichting, E., Skretting, G., Lie, B.A., Sandset, P.M., Iversen, N., 2014. Increased coagulation activity and genetic polymorphisms in the F5, F10 and EPCR genes are associated with breast cancer: a case-control study. BMC Cancer 14, 845.

Wang, H., Xu, X., Pu, C., Li, L., 2019. Clinical characteristics and prognosis of cancer patients with venous thromboembolism. J Cancer Res Ther 15, 344–349.

Xiang, Q., Ji, S.D., Zhang, Z., Zhao, X., Cui, Y.M., 2016. Identification of. Biomed Res Int 2016, 5675084.

Zheng, L.-X., Li, L.i., Huang, J.-H., Mai, Y.-M., Chen, C., Dong, J.-H., Xu, Z.-Y., Li, W.-F., Chen, X.-R., 2021. Venous thromboembolism in patients with severe lung cancer: a narrative review. Annals of Palliative Medicine. 10 (6), 6957–6967.