Defence Life Science Journal, Vol. 7, No. 1, January 2022, pp. 17-26, DOI : 10.14429/dlsj.7.17185

Panel of Regulatory miRNAs for Blood Coagulation under Normoxic and Hypoxic Conditions

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ABSTRACT

Abnormal blood coagulation may lead to venous thromboembolism (VTE), a complex multifactorial disease. Hypoxia (oxygen deprivation) is a major factor disturbing the blood hemostasis and predisposing the body towards coagulation and VTE. Pathophysiology of VTE can be attributed to post-transcriptional gene regulation by microRNAs (miRNAs). The present study identified regulatory miRNAs involved in causing blood coagulation under normoxic and hypoxic conditions. Meta-analysis was performed, following PRISMA guidelines, for identifying miRNAs involved in blood coagulation pathway. Studies evaluating miRNAs from circulating blood as potential biomarkers of VTE were selected. A total of 16 studies met selection criteria and 8 having complete statistical information were selected for analysis. Study of blood coagulation mechanism under hypoxic conditions involved *in-silico* search within highly cited databases to identify miRNAs commonly regulating genes of hypoxia-inducible factor (HIF) family and coagulation pathway. Further bio-informatics approaches were employed to identify potential biomarker candidates. Meta-analysis revealed a panel of 12 miRNAs; two members of miR-27 family, hsa-miR-27a and hsa-miR-27b; two members of miR-320 family, hsa-miR-320a and hsa-miR-320b, hsa-miR-1233, hsa-miR-134, hsa-miR-424-5p, hsa-miR-221, hsa-miR-28-3p, hsa-miR-136-5p, hsa-miR-374-5p and hsa-miR-338-5p involved in blood coagulation under normoxic conditions. Besides these, present *in-silico* analysis identified a set of 5 miRNAs including hsa-miR-4667-5p, hsa-miR-6815-3, hsa-miR-4433a-3p, hsa-miR-6735-5p and hsa-miR-6777-3p which predominantly regulate genes that facilitate both coagulation and response to hypoxic stress. The present study generated a panel of regulatory miRNAs potentially involved in the process of blood coagulation under both normoxic and hypoxic conditions, which may serve as putative epigenetic biomarkers for coagulation.

Keywords: MicroRNA; Hypoxia; Meta-analysis; Coagulation

1. INTRODUCTION

Venous thrombo-embolism (VTE) is a multifactorial blood coagulation disease, which is comprised of two major complications Deep vein thrombosis (DVT), and Pulmonary embolism (PE), which is potentially fatal. Hypoxic environment, especially at high altitudes has been identified as a predisposing factor causing thrombosis. The altered physiological response at hypoxic conditions favors a pro-thrombotic milieu suitable to further accumulate complications like VTE[1]. In alignment with the previous findings, the results demonstrating fibrinogen levels and platelet count with respect to hypoxia reported an increase in fibrinogen levels, which is reasonable, as it is the main component of clot formation.

Insufficient oxygen levels at tissue sites can affect cellular respiration and cause hypoxia. On sensing this decline in the blood oxygen availability by the carotid bodies, the human body undergoes adaptive changes to overcome this stress. This includes activation of regulatory proteins by changes in gene expression, that may rapidly modulate pulmonary ventilation as well as blood circulation, thus promoting survival in oxygen deprivation[2]. There might be several reasons for hypoxia, like hyper-proliferation at tumor sites, classical heart and respiratory diseases like asthma, ascend to high altitude, and sometimes even over-exertion during exercise or physical labor.

In a natural homeostatic balance of the body, the hematological parameters maintain an equilibrium between the anti-coagulants and pro-coagulating factors. However, hypoxic stress exposure can facilitate the activation of the coagulation cascade by increasing thrombin generation and simultaneously increasing Protein C levels and FVIII-mediated thrombin generation[3]. In vitro and animal model studies have also reported hypoxia-induced endothelial dysfunction leading to endothelial cell atrophy, impairment in Ca^{2+} ion homeostasis, and a functional disturbance in eNOS (endothelial nitric oxide synthase) activation[4].

The Hypoxia Inducible Factor (HIF) family of transcription factors are the key regulators facilitating induction of genes for adaption of the body to hypoxia, as well as during optimal environmental conditions[5]. HIF-1 is a heterodimeric protein comprising of two subunits, HIF-1α and HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator (ARNT)). These protein subunits are members of basic Helix-Loop-Helix transcription factor superfamily containing a PAS (PER

Received : 17 June 2021, Revised : 22 December 2021
Accepted : 24 December 2021, Online published : 21 January 2022

(periodic clock)-ARNT-Sim (single minded)) (bHLH-PAS). Five members of this superfamily include, HIF-1a, HIF-2a (Also known as EPAS1 gene), HIF-3a, HIF-1β (ARNT) and ARNT2[6]. Splice variant of HIF-3a, IPAS (Inhibitory-PAS) is a dominant-negative regulator of HIF-1a which revents its DNA binding activity[7]. HIF 1 is a heterodimer of oxygen dependent HIF-1a and constitutively expressed HIF-1b. Post translational regulation of HIF transcription activity occurs by degradation of its subunits under normoxic conditions. However, under hypoxic conditions, such degradation does not take place and HIF heterodimers enter into nucleus where they bind to HRE (Hypoxia Response Element), within the promoter region of large number of genes affecting their transcriptional activity.

There are considerable experimental evidences showing that platelets are activated and their adhesiveness is increased in healthy individuals upon exposure to hypoxia[8]. In accordance to the increase in P-selectin level, the platelet activation biomarker; proteomic profiling of hypoxic platelets also revealed differentially expressed Calpain protein levels[1]; Although the heightened platelet activity cannot be concluded as the sole factor for thrombotic precipitation, this predisposition could be the result of accumulated substrates of coagulation. Since, hypoxia enhances platelet aggregation, which is pivotal to venous thrombosis, this could be an explanation to the fact that venous thrombosis is more prevalent at high altitude compared to arterial thrombosis[9].

In addition, platelet α-granules are a storehouse of key anti-coagulants (like TF, TFPI), pro-coagulant factors (Fibrinogen, FV, FVIII and vWF (von Willebrand factor) and fibrinolytic proteins) which are responsible for coagulation[10]. Interestingly, platelet count has been shown to decrease on exposure to hypoxia, which could be due to increased number of platelets being consumed during the process of aggregation[8]. Findings of a longitudinal study reveal that mean platelet volume and levels of plasma fibrinogen increase on ascent to high altitude[11].

MicroRNAs (miRNAs) belong to class of small RNA epigenetic regulators which control expression of large number of genes by post transcriptional gene silencing. MiRNAs act by hybridizing to target mRNA by non-complimentary base pairing thus affecting its translation and stability. A single miRNA can have multiple mRNA targets[12]. The degree of complementarity between the mRNA and an ~8 nucleotide seed sequence within the ~22 nucleotides stretch of miRNA which binds to the mRNA determines how the later will be silenced; either through translation inhibition or a decrease in mRNA levels[13]. This property of having multiple targets, and altering protein expression post-transcriptionally makes it a strong candidate for target-mediated expressional study as a biomarker for blood coagulation disorders.

This study is an attempt to identify potential miRNA candidate list which regulate post-translational mechanism of gene expression with response to adaptation to hypoxia exposure or under normoxic conditions, as well as regulating genes of the coagulation cascade. It also consolidates all the available literature into a comprehensive meta-analysis to extract essential information from the most relevant findings which align with the objectives of the study. The objective of the present study was to identify common regulatory microRNAs linking Hypoxia and coagulation mechanism. Our findings may pave a way for future experimental validation of these miRNAs to identify biomarkers of hypoxia induced coagulation.

2. METHODOLOGY

The present study involved meta-analysis and *in-silico* analysis of published data and hence ethical approval was not necessary.

2.1 Meta-Analysis

The meta-analysis was performed conforming to guideline laid by Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[14]. A diagrammatic representation of the work-flow is depicted in Fig. 1.

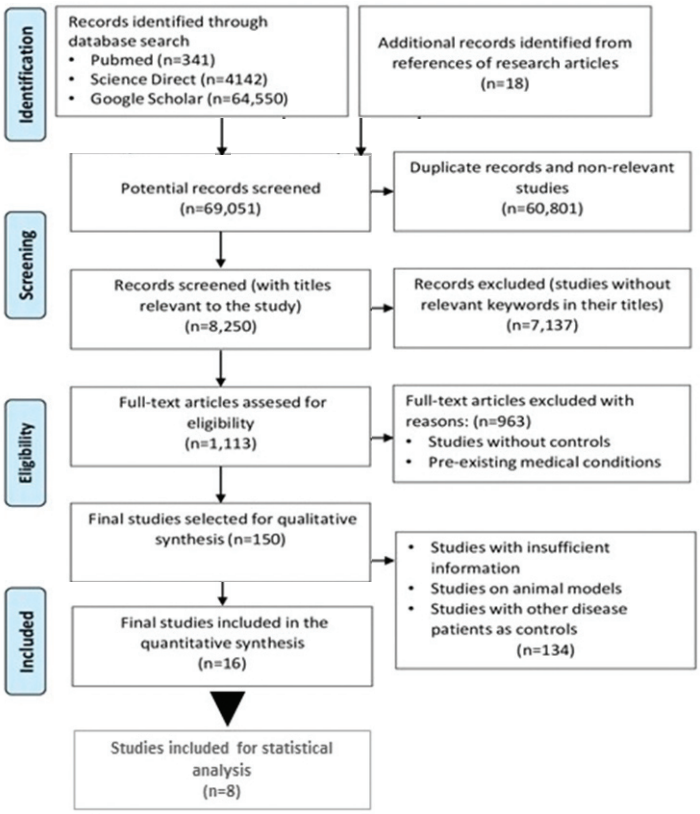

Figure 1. Methodology for meta-analysis, following PRISMA guidelines.

2.1.1 Data Sources and Retrieval Strategy

Literature search was done for all the studies that reported microRNAs in association with VTE, DVT or PE on PubMed, Google Scholar and Science Direct (last search was updated in October 2020). All the databases were searched using common key search terms “microRNA”, “miRNA”, “miR”, “venous thrombosis”, “venous thromboembolism”, “deep vein thrombosis”, “acute pulmonary embolism”, “pulmonary embolism”. A manual search was also done to find relevant articles in previously published meta-analysis and reviews.

2.1.2 Inclusion and Exclusion Criteria

Studies were considered as potentially eligible if they

showed role of microRNAs in the selected pathophysiology. The inclusion criteria for selection of articles were as follows: (i) research articles indicating role of microRNAs in VTE, PE, APE or DVT, (ii) human studies, (iii) association studies, (iv) reports published in English language, (v) full view articles. The excluded studies consisted of reviews, previous meta-analysis, duplicate entries, studies on patients with other co-morbidities, non-heterogenous population studies, and studies without proper sample details.

2.1.3 Study Selection and Data Extraction

The articles that met the inclusion and exclusion criteria were thoroughly read and manually processed for further analysis. The data extracted from the studies included all the details that could be inferred from the articles; (a) first author's name, (b) year of publication, (c) location of study population, (d) sample size, (e) average age, (f) type of cases and controls, (g) source of sample, (h) method of diagnosis, (i) microRNA(s) (j) fold change, (k) p-value, (l) area under curve (AUC), (m) 95 per cent CI, (n) sensitivity, (o) specificity, and (p) standard error.

2.1.4 Statistical Analysis

The statistical analysis of the study was done using Graph pad Prism (5.0) and Comprehensive Meta Analysis Version 3.0 software. The data extracted was analysed for association of microRNAs in VTE and associated complications of DVT and PE by following fixed model effects study after plotting the Forest plot of the data. The study groups were also assessed for heterogeneity, effect size and 95 per cent CI, and 2-tailed test of null.

2.2 In-silico Analysis

2.2.1 Candidate Gene Selection

Second objective of the study was to identify microRNAs involved in coagulation under hypoxic environment. We enlisted 5 hypoxia inducible factor (HIF) family genes as well as genes involved during coagulation, considering only the intrinsic and extrinsic pathway using KEGG pathway database (https://www.genome.jp/kegg/pathway.html).

2.2.2 Data Sources and Retrieval

MiRNA targets were retrieved for genes of HIF family and coagulation pathway from 3 reliable and highly cited databases–miRWalk (http://mirwalk.umm.uni-heidelberg.de/)[15], miRNet (https://www.mirnet.ca/)[16], and miRTargetLink Human (https://ccb-web.cs.uni-saarland.de/mirtargetlink/)[17] without setting up any filters. A vast network data was obtained. Each retrieved dataset from different databases were compiled together and the results were then filtered for any pre-mature miRNAs and repeated entries.

2.2.3 Inclusion Criteria

The data was filtered again exclusively for miRNAs targeting genes of both the HIF family and coagulation pathway. The data was re-organised by sorting the candidate miRNAs with respect to the types and number of targeted genes. This helped us to prioritise the miRNAs targeting most genes of HIF family and coagulation pathway.

2.2.4 Target Identification

We thus obtained a list of candidate miRNAs targeting genes of both the HIF family and coagulation pathway. To validate that these miRNAs are non-ubiquitous in expression, thereby specifically target genes associated with hypoxic adaptation of the body, hemostasis and associated pathways simultaneously; a similar yet opposite approach was implemented on the same previously cited databases to extract details of miRNAs for their target genes.

2.2.5 Target Validation

To confirm that the target gene list predominantly regulated hypoxia associated coagulation cascade by manipulating major biological pathways that assisted the process of thrombus development, the obtained target gene list was categorised according to their ontology and regulated biological pathways. This sorting was performed on ShinyGO[18] and Panther database[19]. A diagrammatic work-flow of the methodology is presented in Fig. 2.

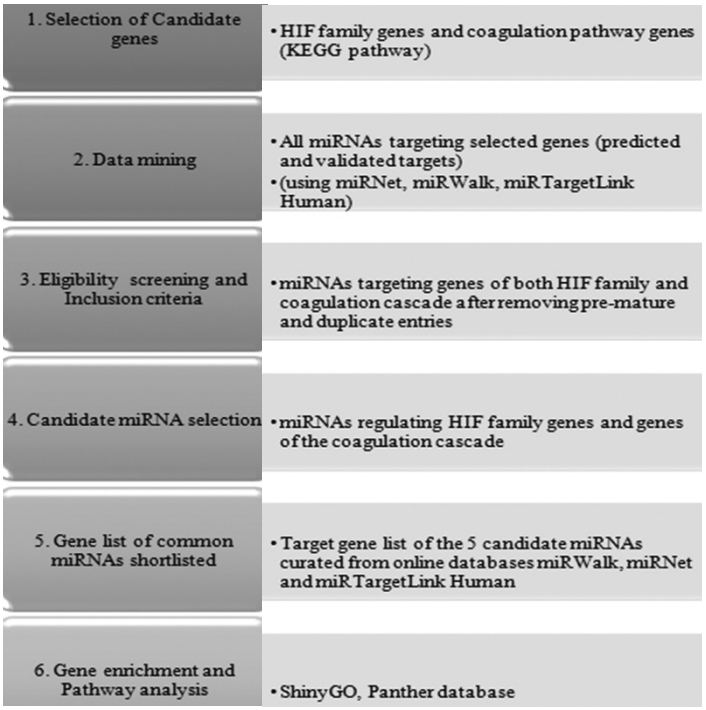

Figure 2. Diagrammatic representation for the work-flow of the in-silico study.

3. RESULTS

3.1 Meta-Analysis

During meta-analysis, a total of 69,051 studies were extracted as an outcome of search through PubMed, Google Scholar and Science Direct database. Out of these, 68,901 publications were excluded, among which 60,801 were duplicate records, 7,137 were non-relevant studies, 963 were studies without control subjects or pre-existing medical conditions, 134 were studies on animal models and studies with other diseases as controls. In the final selection for qualitative analysis, a total of 150 studies were included and scrutinised for details of patients. Studies with insufficient information

were excluded and a total of 16 studies were included for final quantitative synthesis among which 8 studies having complete information were selected for statistical analysis. These studies established the role of miRNAs in promoting VTE, DVT and PE development under normoxic conditions. Among these studies miR-320[20-22] miR-195[23,21,24] and miR-134[25-26] were found to express most frequently in patients.

The final results of the meta-analysis include statistical analysis of results of eight research articles that had all the relevant details needed for the study. Details of the included studies are described in Annexure I. These studies were published between the year 2011 and 2020, and the population under study was mainly from China. All the included studies showed a comparison between the patients (APE, PE or DVT) and healthy controls. The sample used for estimating miRNA levels was Peripheral blood mononuclear cells (PBMCs), serum or plasma.

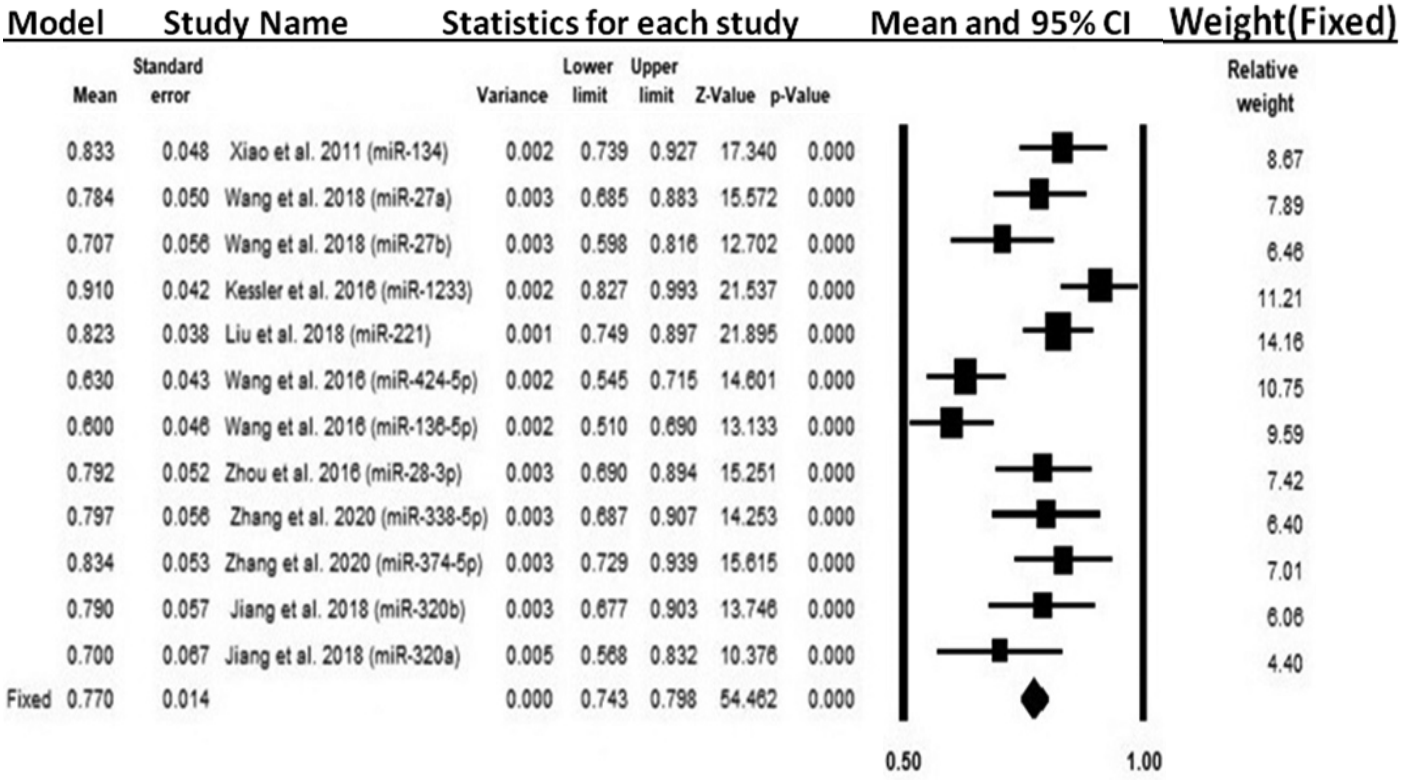

Figure 3. Forest plot analysis of the selected studies in a fixed effect model.

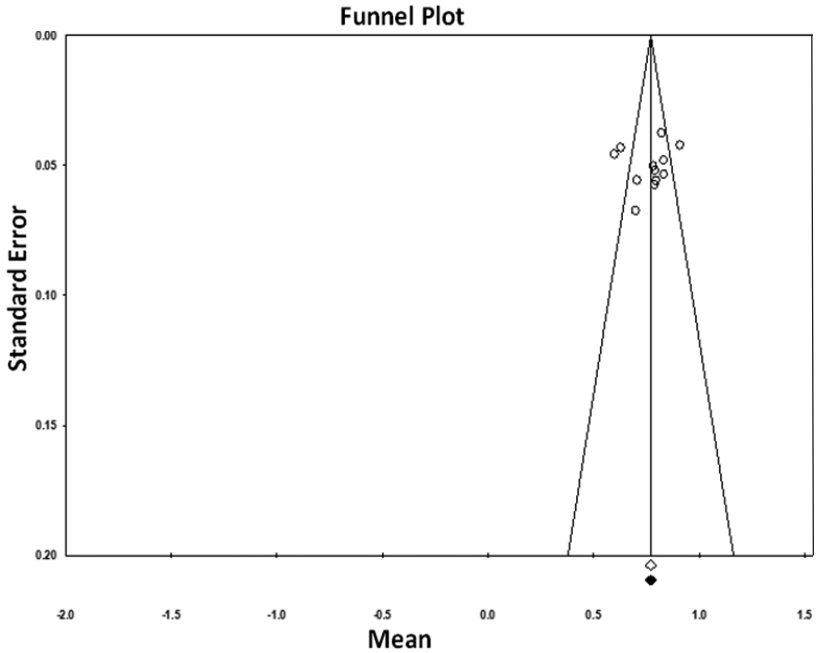

Figure 4. Funnel plot of standard error by mean.

The statistical analysis of the eight studies and their relative weight for the forest plot point estimate of the averaged studies lies far right to the line of null effect, depicting the all the included studies showed an association of miRNA in the subjects, with the fixed model averaged mean value of 0.77 and standard error 0.014 (Fig. 3). This was also later confirmed by the effect size and 95 per cent CI value point size estimate of 0.77. The Funnel plot of standard error by mean showed some asymmetry in the data, however this small study effect might be due to chance alone (Fig. 4). The statistical analysis of the study data also rejected the null-hypothesis by 2-tailed test Z-value of 54.462. The test for heterogeneity Q-value and I^2 value was 43.466 and 74.683, however these large variations might be within the study subjects within the selected study groups (Annexure II).

3.2 In *silico* Analysis

In the *in-silico* analysis, the study for miRNAs

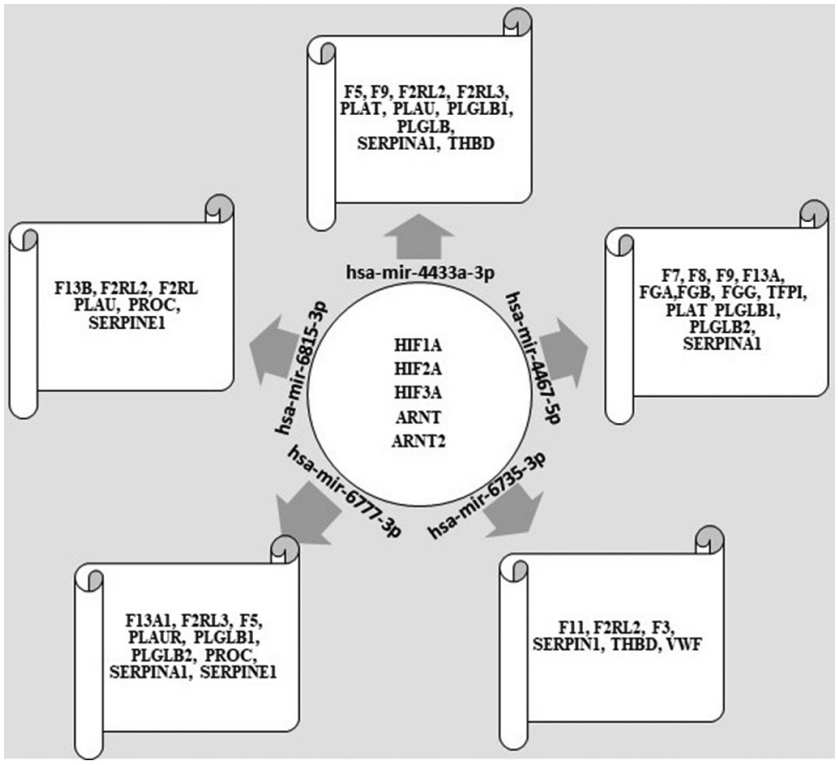

Figure 5. The candidate microRNAs (miR-4667-5p, miR-4433a-3p, miR-6777-3p, miR-6815-3p and miR-6735-5p) and their respective targets of hypoxia and coagulation pathway genes.

Table 1. Other candidate miRNAs list prioritized on the basis of number and type hypoxia and coagulation pathway genes regulated by them.

MicroRNAs	HIF Family Genes	Coagulation Pathway Genes
miR-5698 miR-4633-3p miR-504-5p miR-6782-5p miR-6893-5p	ARNT, ARNT2, HIF1A, HIF3A	F2, F3, F8, F9, F11, F2R, F2RL1, F2RL2, F2RL3, FGB, PLAT, PLAUR, PROCR, TFPI, PLG, PLGLB1, PLGLB2, THBD, CPB2, KNG1, KLKB1, SERPINA1, SERPINE1
miR-6510-5p	ARNT, HIF1A, HIF2A, HIF3A	F13B, F2RL2, F5, F8, F9, FGA, FGB, KNG1, PLGLB1, PLGLB2, PROCR, PROS1, SERPINA1, SERPINE1
miR-3157-5p	ARNT2, HIF1A, HIF2A, HIF3A	F2RL1, PLGLB1, PLGLB2, PROS1
miR-4701-3p	ARNT, ARNT2, HIF1A, HIF2A	F13A1, F5, FGB, PLAT, PLGLB1, PLGLB2, PROCR, SERPINA1, TFPI

regulating HIF family genes and that of the coagulation cascade under hypoxic conditions was conducted and a systematic approach was applied to the predict candidate miRNAs whose target genes overlap between regulatory networks of blood coagulation and hypoxic adaptation. On validation of gene targets of the candidate miRNAs for non-ubiquitous expression and targets within the interests of the aim of study, it was confirmed that the identified microRNA candidates of the *in-silico* analysis specifically regulated genes essential for adaptation to hypoxia and associated biological pathways. In addition, other associated signaling pathways complementing thrombus formation like plasminogen activation cascade, and endothelin signaling were also predominantly involved.

3.2.1 miRNAs Eegulating HIF Family Genes

Under this approach, the five genes of HIF family were selected which included, (i)HIF1a, (ii) HIF2a, (iii)HIF3a, (iv)ARNT and (v)ARNT2. The initial database search for miRNAs targeting these genes enlisted over 2300 miRNA entries. Enlisting of miRNAs was followed removal of pre-mature miRNAs and duplicate entries after sorting.

3.2.2 miRNAs Regulating Coagulation Pathway Genes

The selection procedure of miRNAs was similar as previously described, including all coagulation genes, receptors and their mediators. Some genes only enlisted a few miRNAs. However, all the entries for validated and predicted miRNA targets from the three databases were included for a total 42 coagulation pathway genes.

3.2.3 miRNAs Commonly Regulating Hypoxia and Coagulation Pathway Genes

MicroRNAs common between HIF family and coagulation cascade genes were selected, whereas the ones selectively targeting only either of the groups were excluded from further analysis. After extensive sorting, a list of 1445 miRNA entries were registered. The data was further grouped on the basis of types and number of genes involved in both the pathways (Annexure II). This list was then prioritised on the basis of most HIF genes regulated; this was necessary for candidate miRNA selection for miRNA-gene network interaction study.

At the end of this comprehensive sorting, a panel of 5 prioritised candidate miRNAs including miR-4667-5p, miR-4433a-3p, miR-6777-3p, miR-6815-3p and miR-6735-5p was obtained (Fig. 5). Apart from these 5 candidate miRNAs listed, other miRNAs which also regulated at least four genes of the HIF family and other important coagulation pathway genes were also manually extracted during the process (Table 1).

3.2.4 Gene Annotation and Pathway Analysis

The target genes of prioritised miRNAs were validated by studying gene annotation using ShinyGO database which classified the genes into biological processes, molecular functions and cellular components. Pathways analysis done using Panther database yielded a list of major related

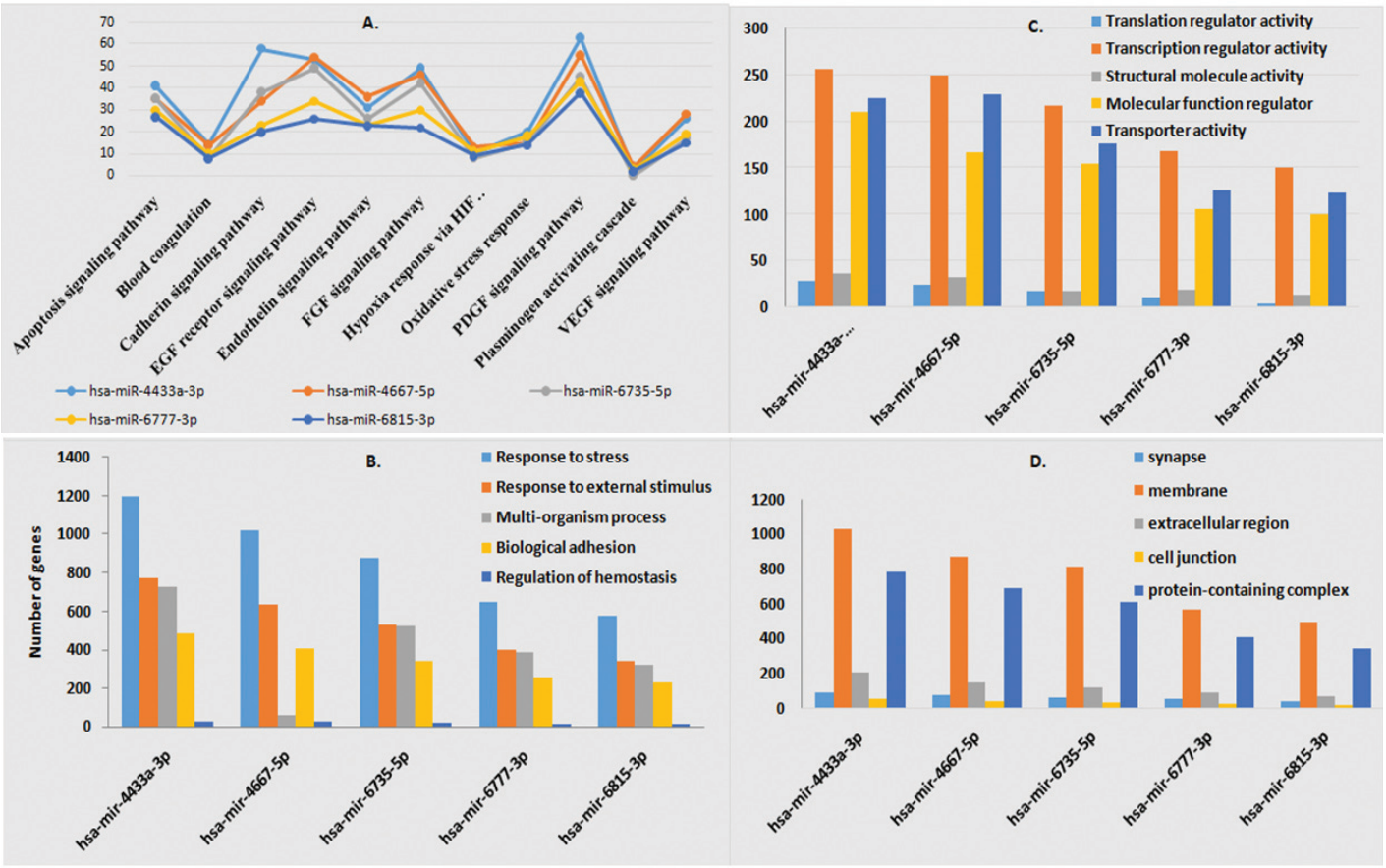

Figure 6. Gene enrichment and pathway analysis of 5 miRNAs using ShinyGO and Panther database ((A. Line graph showing number of genes targeted by miRNAs in selected Biological pathways, B. Histogram of key Biological process, C. Histogram of key Molecular functions, D. Histogram showing important Cellular components).

pathways involved in regulatory processes such as maintaining homeostasis, response to stress and other multi-organism processes (Fig. 6).

An insight into these biological processes particularised a diversity of signaling cascades, including special emphasis on hypoxia response via HIF activation, coagulation, PDGF signaling and plasminogen activation cascade. Complementary pathways like VEGF signaling, oxidative stress response and endothelin signaling pathway were also regulated. Among the predominantly regulated biological processes were regulation of hemostasis, response to stress, biological adhesion and other multi-organism process and their regulation. Molecular functions included transcription/translation regulator activity, catalytic and transporter activities. Similarly, cellular components like organelle, membrane, cell, protein containing complexes and extracellular regions were also regulated (Fig. 6).

4. DISCUSSION

A conclusive study of meta-analysis of the previously published articles reporting role of microRNAs in VTE and associated pathophysiology under normoxic conditions was conducted. A total of 12 miRNAs; miR-27a/b, miR-320a/b, miR-1233, miRNA-134, miR-424-5p, miR-221, miR-136-5p, miR-28-3p, miR-374-5p and miR-338-5p and are presented as potential candidates to be used as biomarkers for VTE diagnosis. These miRNAs have been accounted for critical role in thrombosis and showing differential expression levels. MicroRNA-134 was validated in patients and controls of acute pulmonary embolism (APE), and the expression was found to be increased in patients with a fold change of 25.392[25]. Wang et. al studied the expression level of miR-27a and miR-27b in 148 APE patients. The study reports upregulated expression of miR-27a/b with a sensitivity and specificity of 0.792 and 0.7 of miR-27a and 0.646 and 0.775 for miR-27b respectively[27]. Similarly, Kessler et. al also report an upregulated level of miR-1233 in PE patients with a sensitivity and specificity of 0.9 and 0.92 respectively[28]. MiR-221 showed a fold change of 4 in the serum of APE patients[29]. Wang et. al 2016 included the largest number of DVT patients with an miR-424-5p and miR-136-5p fold change of 1.87 and -2.22 respectively[30]. MiR-28-3p studied in PE patients of in by Zhou and co-workers reported a 1.66-fold change while the sensitivity and specificity were 0.62 and 0.83 respectively[31]. Jiang and coworkers reported 1.79- and 1.58-fold change in miR-320a and miR-320b respectively[20]. The most recent study conducted on DVT patients for deferential expression of miR-338-5p and miR-374-5p also report an AUC value of 0.797 and 0.834 respectively[32].

During conditions of physiological stress, such as hypoxia, especially at high altitudes, the body is more susceptible to a prothrombotic state. Hypoxia triggers the activation of the coagulation cascade genes regardless of the physical activity[33]. A number of studies have reported that hypoxia predisposes

an individual towards hemostasis. Disturbance of equilibrium between pro-coagulants and anti-coagulants may result in blood clotting disorders like stroke, DVT, PE. It is a well-established fact that HIF regulates a wide variety of genes[34] which are particularly responsible for body's adaptation to hypoxia. Also, since microRNAs are known as key modulators of gene expression and they could be of considerable importance in regulating mechanism of HIF genes expression.

In an attempt to establish a common regulatory link between hypoxia and coagulation pathway, this systematic *in-silico* analysis consolidates a list of microRNAs which overlap in function between both the biological pathways. Since only a handful of research cite the association of miRNAs in hypoxia induced coagulation[21], a thorough search of highly cited databases like miRWalk, mirTargetLink Human and miRNet followed by enrichment analysis yielded a panel of five microRNAs, miR-4667-5p, miR-4433a-3p, miR-6777-3p, miR-6815-3p and miR-6735-5p as potential regulatory biomarkers. To further validate the findings of the study, a reverse approach was used to find all the target genes and a Gene Ontology and biological pathway analysis was done with the help of Panther and ShinyGO databases. The findings of the study corroborate our hypothesis, as the genes regulating major biological processes like hemostasis, biological response to stress and adhesion were not ubiquitously expressed. They were also shown to regulate biological pathways like blood coagulation, hypoxia response via HIF activation, PDGF signaling, and thus predominantly facilitated the coagulation cascade. Therefore, this study contemplates a thoroughly curated list of microRNAs which regulate both hypoxia and coagulation pathway, including associated supporting pathways facilitating thrombus formation in hypoxic conditions. The predicted miRNA candidates may be further used as reference to extrapolate these findings in cell lines and animal models. This shall implicate their role as diagnostic biomarkers and therapeutic targets for blood coagulation mechanism under normoxic and hypoxic conditions.

Present study gives two separate miRNA panels and did not find any common miRNA between the two panels suggested for blood coagulation under normoxic and hypoxic conditions. A few review reports have previously emphasised upon the role of miRNAs as diagnostic biomarkers in VTE[35]. However, in addition to the previous findings, our study simultaneously conducted an *in-silico* analysis and a meta-analysis to infer all the candidate miRNAs that have been previously studied in association with VTE, DVT and PE under normoxic as well as hypoxic environment conditions.

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Her contribution in the current study involved conceptualisation & manuscript proof reading.

Annexure I

Details of studies included for statistical analysis.

Author	miRNA	Location	Sample size	Average age	Type	FC	p-value	AUC	95% CI(Lower)	95% CI (Upper)	Sensitivity	Specificity	SE
Xiao *et al.* 2011	miR-134	China	64	54.78±16.20	APE vs controls	25.392	0.047	0.833	0.737	0.929	-	-	-
Wang *et al.* 2018	miR-27a	China	148	61.0±11.9	APE vs controls	-	<0.001	0.784	0.685	0.884	0.792	0.7	0.051
Wang *et al.* 2018	miR-27b	China	148	61.0±11.9	APE vs controls	-	<0.001	0.707	0.597	0.817	0.646	0.775	0.056
Kessler *et al.* 2016	miR-1233	Germany	48	62.0±14	PE vs controls	-	<0.001	0.91	0.82	0.99	0.9	0.92	-
Liu *et al.* 2018	miR-221	China	110	55.83±7.52	APE vs controls	4	<0.05	0.823	0.757	0.906	-	-	-
Wang *et al.* 2016	miR-424-5p	Sweden	238	59.8±19.1	DVT vs controls	1.87	0.01	0.63	0.54	0.71	0.31	0.62	-
Wang *et al.* 2016	miR-136-5p	Sweden	238	59.8±19.1	DVT vs controls	-2.22	0.03	0.6	0.51	0.69	0.31	0.61	-
Zhou *et al.* 2016	miR-28-3p	China	74	42.0±11	PE vs controls	1.66	<0.05	0.792	0.689	0.896	0.62	0.83	-
Zhang *et al.* 2020	miR-338-5p	China	72	57.3±9.9	DVT vs controls	-	-	0.797	0.685	0.908	-	-	-
Zhang *et al.* 2020	miR-374-5p	China	60	NA	DVT vs controls		<0.05	0.834	0.728	0.941	-	-	-
Jiang *et al.* 2018	miR-320b	China	60	52.56±15.42	DVT vs controls	1.79	<0.0001	0.79	0.67	0.9	-	-	-
Jiang *et al.* 2018	miR-320a	China	60	52.56±15.42	DVT vs controls	1.58	0.003	0.7	0.56	0.83	-	-	-

Annexure II

Statistical analysis of the selected studies

		Effect size and 95% confidence interval					Test of null (2-Tailed)		Heterogeneity				Tau-squared			
Model	No. of studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df(Q)	P-value	I-squared	Tau-squared	Standard Error	Variance	Tau
Fixed	12	0.77	0.014	0	0.743	0.798	54.462	0	43.466	11	0	74.693	0.007	0.004	0	0.085