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Incidence of thrombophilic gene polymorphism (MTHFR C677T) in Egyptian COVID-19 patients and its clinical implications

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Abstract

Background COVID-19 has an important component of organ damage which is COVID-19-associated coagulopathy. It is necessary to assess the risk in patients to develop a thrombophylaxis plan. The higher prevalence of key thrombophilic genetic variants, such as mutation of the C677T-methylenetetrahydrofolate reductase (MTHFR) gene in Eastern Mediterranean countries, makes it challenging to use the same criteria in other world countries with differing thrombophilic panels.

Objective To find the incidence of MTHFR gene polymorphism in a cohort of Egyptian patients with COVID-19, and its association with thromboembolic events.

Subjects and methods This was a prospective observational cohort study, done at Ain-Shams University isolation Hospitals, Cairo, Egypt. It included 33 patients with COVID-19 and 13 healthy controls. The patients underwent lab investigations: HRCT chest in which the extent of radiological affection was described in terms of severe form (> 50% of lungs are affected) and non-severe form (< 50% of lungs are affected) and assessment of MTHFR-C677T genotypes. Then follow-up for 28 days for vascular thrombotic manifestations.

Results Out of 33 patients, MTHFR-gene mutation was found in 10 (incidence rate 30.3%). Severe form of affection in the HRCT chest was significantly related to mutation of the MTHFR gene (*P* value = 0.009). Patient cure and discharge were significantly related to the absence of mutation of MTHFR-gene (*P* value = 0.025), whereas death and radiological evidence of thrombosis were significantly related to the presence of MTHFR-gene mutation (*P* value = 0.027 and 0.022 respectively). Age > 55 years (60% sensitivity, 100% specificity, PPV 100%), albumin ≤ 3.2 gm/dl (50% sensitivity, 95.65% specificity, PPV83.3%), and ferritin > 453 ng/L (70% sensitivity, 82.61% specificity, PPV 63.6%) were predictors of mutation of MTHFR-gene.

Conclusion Incidence of mutation of MTHFR-gene was 30.3% in COVID-19 patients. Results suggest a potential association between inherited MTHFR gene mutation and severe form of COVID-19, thromboembolic events, and mortality.

Trial registration ClinicalTrials.gov ID: NCT05679414. https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S000CU2V&selectaction=Edit&uid=U00056R5&ts=2&cx=lrrb7q. Retrospectively registered. 9th Jan 2023.

Keywords COVID-19, Egypt, MTHFR gene mutation, Thromboembolism, Outcome

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Background

Due to the persistence of a hypercoagulability state, infection by SARS-CoV-2 is linked to numerous microvascular and macrovascular thrombotic events known as "COVID-19-associated coagulopathy" (CAC), which contributes significantly to organ damage in COVID 19. Up to one-third of these patients experience thrombotic events, which are linked to worsening disease severity and higher mortality [1].

As a result, COVID-19 patients require risk stratification to develop an appropriate thrombophylaxis plan. There have been numerous recommended methods and scores, including those from the American Society of Hematology [2] and the International Society on Thrombosis and Haemostasis [3].

Applying the same standards to other world areas with various pro-thrombotic characteristics is challenging. Though, compared to other regions of the world, the Eastern Mediterranean region has an increased prevalence of major mutations in thrombophilic genes, of them the mutation in C677T methylenetetrahydrofolate reductase (MTHFR) gene [4], with 11% of the population having the homozygous mutation of the C677T genotype [5]. A crucial enzyme in the metabolism of homocysteine, (MTHFR) produces the 5-methyl-tetrahydrofolate, which is present in the blood and provides methyl groups, so homocysteine changed to methionine by re-methylation [6]. A modest increase in the risk of thrombosis is linked to hyperhomocysteinemia, which results from MTHFR's reduced activity [7].

The MTHFR gene contains a common autosomal recessive mutation (C677T) that arises when alanine is changed into the amino acid valine. A higher risk of emerging cardiovascular illness, Alzheimer's disease, depressive disorders, and neural tube abnormalities have been linked to the common C677T variant in MTHFR [8]. Additionally, compared to a heterozygous mutation, a homozygous mutation of that gene creates a higher thrombosis risk in patients [9]. Therefore, a thorough clinical and laboratory assessment should be used to determine the risk of thrombosis as well as the best anticoagulation strategy.

Objective

To find the incidence of MTHFR gene polymorphism in a cohort of Egyptian patients with COVID-19, and its association with thromboembolic events and outcome.

Subjects and methods

Study design

Prospective observational cohort study.

Study setting

The study was done at Ain-Shams University Isolation Hospitals, Cairo, Egypt, started on 1st October 2021 and ended on 31st March 2022.

Study population

Study arm

Thirty-three COVID-19 patients and positive reverse transcriptase polymerase chain reaction (RT-PCR).

Control arm

Thirteen healthy individuals (no clinical evidence of COVID-19 infection).

Patients underwent the following:

1. Medical history and examination.
2. RT- PCR for SARS-CoV2
3. Lab investigations: complete blood count (CBC), C reactive protein (CRP), serum Ferritin, D-dimers, lactate dehydrogenase (LDH)
4. HRCT chest in which the extent of radiological affection was described in terms of severe form (> 50% of lungs are affected) and non-severe form (< 50% of lungs are affected) [10].
5. COVID-19 disease severity classification of the study group according to WHO Living guidance for clinical management of COVID-19, 2021 [11].
6. The patient receives their standard of care treatment in accordance with the patient management protocol of the Egyptian Ministry of Health and Population (MOHP) for COVID-19 Sept. 2021 for Version 1.5 [10].
7. Testing RT-PCR of MTHFR-C677T genotypes using 2 ml EDTA blood.
8. Observe the patient for 28 days for the detection of vascular thrombotic manifestation (possible clinical data, lower limb duplex, rising D-dimer, or if necessary computed tomography of the chest using pulmonary angiography).
9. Follow the patient for 28 days to assess the patient's outcome (cure or death).

Criteria of inclusion

Adult COVID-19 patients, ≥ 18 years with positive RT-PCR.

Criteria of exclusion

- Patients' refusion of study participation.
- Patients aged less than 18 years old
- Patients on antithrombotic treatment prior to COVID-19.
- Patients with established blood coagulation disorders

Sample size

A sample size was calculated to recruit 33 patients with COVID-19 admitted at Ain-Shams University isolation Hospitals in addition to 13 healthy controls.

Sampling method

To get the study sample, convenient sampling was employed. Until the study's sample size was reached, all patients who met the inclusion criteria were enrolled in it. There was no sample storage and no leftovers.

Assessment of MTHFR-C677T genotypes

The real-time PCR using a PCR kit was intended to identify the C677T-MTHFR gene mutation. Its technique relies on probes labeled by fluorophore which is specific for alleles to detect a sequence of the target amplification and detection. A single nucleotide polymorphism (C677T) exists between the cytosine and thymine bases. is the target sequence. In the FAM fluorescence channel, the wild-type allele (C677C) is found, and The HEX fluorescence channel has the mutant allele (T677T). A signal can be seen in both channels if the genotype is heterozygous (C677T). The kit specific for detection uses "hot start" technology and incorporates Ready to Use MasterMix to minimize reactions that are not specific and ensure it is optimally sensitive. Kit is intended for in vitro diagnosis.

Purification of nucleic acid consistent with the clinical material isolation protocols, nucleic acid isolation should be carried out using isolation kits that are readily accessible on the market. The following isolation kits are suggested by the manufacturer: croBEE NA16 Nucleic Acid Extraction System Gene Proof Pathogen Free DNA Isolation KitPCR set up:

1. Fill the PCR tubes with 18 µl of MasterMix.
2. Fill each PCR tube with 2 µl of the isolated nucleic acid sample or µl of the positive control. Twenty microliters will be the total volume of the reaction mix. During the PCR preparation, all components must be kept between + 2 and + 8 °C.
3. The tubes should be sealed, quickly centrifuged, inserted into the instrument, and allowed to amplify using the following PCR profile.

Statistical analysis methodology

Version 23 of the Statistical Package for the Social Sciences (IBM SPSS) was produced in Hong Kong, China, and used for data collection, revision, coding, and entry. Where the data were found to be parametric, they were presented as mean, SDs, and ranges; where they were nonparametric, the interquartile range and median were given. Numbers and percentages were also used to display qualitative variables. Using qualitative data, groups were compared using the 2 tests. Two groups with quantitative data and a nonparametric distribution were compared using the Mann–Whitney test, while two groups with quantitative data and a parametric distribution were compared using an independent *t*-test. The 95% confidence interval and the permitted margin of error were both set at 5%. The odds ratios (OR) and associated 95% confidence intervals (CI) for both univariate and multivariate logistic regression models were evaluated. This led to the *P* value being classified as significant in the following ways: *P* values less than 0.05 are considered significant, and those less than 0.01 are highly significant.

Ethical consideration

Consent to participate and ethical approval upon receiving assurances of confidentiality, each patient who was invited to take part in the study provided written informed consent. They had the option to withdraw from the study at any time, either on their own or with the consent of their legal guardian; in this case, they continued to be monitored and treated as needed. The ethics committee of scientific research, Faculty of Medicine, Ain Shams University, gave its approval. (REC number: FMASU R 130/2022).

Results

The current study included 33 Covid 19 patients and 13 healthy controls. In the study group, 19 patients were males and 14 were females with a mean age of 45.42 ± 10.56 years. In the control group, 6 subjects were males and 7 were females with a mean age of 34.85 ± 13.13 years.

Table 1 displays descriptive data about the study group's age (years), sex, MTHFR gene mutation, smoking status, symptoms and their duration, and comorbidities. Out of 33 patients, a heterozygous form of MTHFR-gene mutation was found in 9 (27.3%), homozygous form was found in 1 (3.0%).

Table 2 displays laboratory results for the study group for serum IL-6, CRP, albumin, ferritin, D dimer, TLC, and lymphocyte count.

The clinical course of the study group regarding vital data, the need for mechanical ventilation, site of care, disease severity classification, HRCT chest radiological affection, and outcome (cured/discharged, continued hospitalization, death, duration of hospital admission, and radiological evidence of thrombosis) are shown in Table 3.

Table 1 Descriptive data of the study group regarding age (years), sex, MTHFR gene mutation, smoking status, symptoms and their duration, and comorbidities

		No. = 33
Age (years)	Mean ± SD	45.42 ± 10.56
	Range	25–67
Sex [*N*° (%)]	Female	14 (42.4%)
	Male	19 (57.6%)
MTHFR gene mutation [*N*° (%)]	Absent	23 (69.7%)
	Heterozygous	9 (27.3%)
	Homozygous	1 (3.0%)
Smoking [*N*° (%)]	Non-smoker	22 (66.7%)
	Smoker	11 (33.3%)
Symptoms [*N*° (%)]		
Fever	No	14 (42.4%)
	Yes	19 (57.6%)
Fatigue	No	1 (3.0%)
	Yes	32 (97.0%)
Bone ache	No	3 (9.1%)
	Yes	30 (90.9%)
Anosmia	No	21 (63.6%)
	Yes	12 (36.4%)
Nausea or vomiting	No	17 (51.5%)
	Yes	16 (48.5%)
Diarrhea	No	21 (63.6%)
	Yes	12 (36.4%)
Abdominal pain	No	21 (63.6%)
	Yes	12 (36.4%)
Sore throat	No	21 (63.6%)
	Yes	12 (36.4%)
Cough	No	19 (57.6%)
	Yes	14 (42.4%)
Dyspnea	No	15 (45.5%)
	Yes	18 (54.5%)
Co-morbidities [*N*° (%)]		
Diabetes	No	23 (69.7%)
	Yes	10 (30.3%)
Hypertension	No	27 (81.8%)
	Yes	6 (18.2%)
Ischemic heart disease	No	28 (84.8%)
	Yes	5 (15.2%)
Chronic chest diseases	No	29 (87.9%)
	Yes	4 (12.1%)
Duration of symptoms (days)	Median (IQR)	3 (3–4)
	Range	1–14

MTHFR methylenetetrahydrofolate reductase, *SD* Standard deviation, *IQR* Interquartile range

Table 2 The study group's laboratory results as regards serum IL-6, CRP, albumin, ferritin, D dimer, TLC, and lymphocyte count

		No. = 33
Laboratory parameters		
Serum IL-6 (ng/L) *Normal value is up to 7 ng/l*	Median (IQR)	29 (19–46)
	Range	3–126
CRP (mg/L) *Normal value (*up to 0.50 mg/L*)*	Median (IQR)	16 (8.3–23)
	Range	0.16–116
Albumin (g/dL) Norman value (3.4–5.4 g/dl)	Mean ± SD	3.62 ± 0.38
	Range	2.6–4
Ferritin (ng/ml) *Normal value* (13.0–150.0 ng/ml)	Median (IQR)	343 (212–534)
	Range	6–2000
D dimer (mg/l) *Normal value is* up to 0.55 mg/l	Median (IQR)	0.65 (0.55–0.78)
	Range	0.08–2.1
TLC (10^3/μL) *Normal value is (*4000–11,00 (10^3/μL)	Mean ± SD	6.41 ± 2.32
	Range	3–12.3
Lymphocytes (10^3/μL) (1.0–3.0 × 10^3/μL)	Mean ± SD	1.32 ± 0.54
	Range	0.6–2.8

IL-6 Interleukin 6, *CRP* C-reactive protein, *TLC* Total leucocytic count, *IQR* Interquartile range, *SD* Standard deviation

Table 3 The study group's clinical data regarding vital data, the need for mechanical ventilation, site of care, disease severity classification, HRCT chest radiological affection, standard of care treatment, outcome, and radiological evidence of thrombosis

Vital data		**No. = 33**
Respiratory rate	Mean ± SD	21.12 ± 5.76
	Range	16–38
SpO_2 (%)	Mean ± SD	91.58 ± 10.73
	Range	60–99
Need for mechanical ventilation [*n* (%)]	No	30 (90.9%)
	Yes	3 (9.1%)
Site of care: Ward/ ICU [n (%)]	ward	28 (84.8%)
	ICU	5 (15.2%)
Disease severity [*n* (%)]	Mild	14 (42.4%)
	Moderate to severe	14 (42.4%)
	Critical	5 (15.2%)
HRCT chest findings [*n* (%)]	< 50%	28 (84.8%)
	> 50%	5 (15.2%)
Outcome [*n* (%)]		
Cured/discharged	30	(90.9%)
Continued hospitalization	1	(3.03%)
Death	2	(6.06%)
Duration of hospital admission	Median (IQR)	15 (12–30)
	Range	6–120
Radiological evidence of thrombosis	No	30 (90.9%)
	PE	2 (6.1%)
	DVT	1 (3.0%)

SpO2 (%) Peripheral oxygen saturation, *ICU* Intensive care unit, *HRCT* High-resolution computed tomography, *PE* Pulmonary embolism, *DVT* Deep venous thrombosis, *IQR* Interquartile range, *SD* Standard deviation

Table 4 Comparison between the study and control groups as regards age, sex, and MTHFR gene mutation

		Control group No. = 13	Patients group No. = 33	Test value	*P*-value	Sig
Age [*n* (%)]	Mean ± SD	34.85 ± 13.13	45.42 ± 10.56	−2.853[b]	0.007	HS
	Range	20–64	25–67			
Sex [*n* (%)]	Female	7 (53.8%)	14 (42.4%)	0.490[a]	0.484	NS
	Male	6 (46.2%)	19 (57.6%)			
MTHFR gene mutation [*n* (%)]	Absent	8 (61.5%)	23 (69.7%)	0.870[a]	0.647	NS
	Heterozygous	5 (38.5%)	9 (27.3%)			
	Homozygous	0 (0.0%)	1 (3.0%)			

P value > *0.05* non-significant (NS); *P value* < *0.05* significant (S); *P value* < *0.01* highly significant (HS)

[a] Chi-square test

[b] Independent *t*-test, *IQR* interquartile range

Age was statistically higher in the study group, as displayed in Table 4, when age, sex, and mutation of the MTHFR gene were compared in the 2 groups.

This study found that among clinical factors associated with the mutation of the MTHFR gene, age, diarrhea, abdominal pain, diabetes, and hypertension were significantly related to the MTHFR gene mutation. Also, radiological affection > 50% in the HRCT chest showed the same relation as displayed in Table 5. Regarding the laboratory data associated with the presence of MTHFR gene mutation, lower levels of albumin and higher ferritin levels were significantly associated with the mutation of the MTHFR gene as displayed in Table 6.

Patient cure and discharge were significantly related to the absence of MTHFR gene mutation, on the other hand, death and radiological evidence of thrombosis were significantly related to the mutation of the MTHFR gene as Table 7 illustrates.

ROC curve of age, albumin level, and ferritin level as predictors of MTHFR gene mutation revealed that: age > 55 years showed 60% sensitivity, 100% specificity with 100% PPV, Albumin ≤ 3.2 gm/dl showed 50% sensitivity, 95.65% specificity with 83.3% PPV. Lastly, Ferritin > 453 ng/L showed 70% sensitivity, and 82.61% specificity with 63.6% PPV as detailed in Table 8 and illustrated in Fig. 1.

Regarding the 13 control healthy individuals: 5 (38.4%) of them the expressed presence of the MTHFR gene heterozygous mutation with no thromboembolic disease manifestation detected.

Discussion

In the metabolism of folate, MTHFR is a crucial enzyme. The enzyme in a "thermolabile" form that was marginally less efficient was identified in 1988 [12]. The polymorphism that caused this, a nucleotide 677 C > T transition, was identified in 1995 [13]. A1298C transition, a further polymorphism with diminished activity had been identified shortly after [14]. According to demographic research, these polymorphisms are quite prevalent, with 60% to 70% of the population carrying just one variant. In fact, homozygous polymorphisms or compound heterozygotes were discovered in 10% of the population [15]. Studies showed that individuals with this polymorphism had increased homocysteine levels as soon as they were discovered. Later studies hypothesized a connection between thromboembolism and the existence of these polymorphisms [16]. Performing MTHRF mutation assays soon joined the list of reliable "classic tests" for thrombophilia, Antithrombin activity, factor V Leiden assays, and other circumstances that have all been conclusively related to a higher risk of VTE. It was undoubtedly an interesting topic about a common mutation that causes high homocysteine levels to lead to thrombosis [17].

Since MTHFR mutation raises serum homocysteine in low folate situations, folate supplementation of grain products was made mandatory in nations like the USA in 1996 [18, 19].

The cytokine storm-like COVID-19 coagulopathy, which manifests as hyperinflammation, coagulation, and platelet activation, is thought to be the result of interactions between the immune and inflammatory systems and the coagulation system. VTE (and particularly pulmonary embolism) was of high incidence when compared to historical controls, as well as in situ pulmonary embolism associated with microthrombi, suggested a classic macro vessel disease as well as thrombotic microangiopathic process; and critically ill COVID-19 patients most significantly had a high rate of VTE [2].

Inherited thrombophilia may contribute to the increased risk of thrombosis in people with COVID-19, according to various reviews or editorials [20]. However, a review of the bibliographical sources from PubMed (January 2023) using the phrases "COVID-19" and "thrombosis" reveals that there is a small number of research that discusses congenital thrombophilia [21].

Table 5 Clinical variables linked to the MTHFR gene mutation in the study group

[*n* (%)]		MTHFR gene mutation		Test value	*P* value	Sig
		Absent	Present			
		No. = 23	No. = 10			
Age	Mean ± SD	42.70 ± 8.39	51.70 ± 12.72	−2.414[b]	0.022	S
	Range	25–55	29–67			
Sex	Female	11 (47.8%)	3 (30.0%)	0.907[a]	0.341	NS
	Male	12 (52.2%)	7 (70.0%)			
Smoking	Non-smoker	17 (73.9%)	5 (50.0%)	1.793[a]	0.181	NS
	Smoker	6 (26.1%)	5 (50.0%)			
Fever	No	10 (43.5%)	4 (40.0%)	0.035[a]	0.853	NS
	Yes	13 (56.5%)	6 (60.0%)			
Fatigue	No	1 (4.3%)	0 (0.0%)	0.448[a]	0.503	NS
	Yes	22 (95.7%)	10 (100.0%)			
Bone ache	No	0 (0.0%)	3 (30.0%)	7.590[a]	0.006	HS
	Yes	23 (100.0%)	7 (70.0%)			
Anosmia	No	14 (60.9%)	7 (70.0%)	0.251[a]	0.616	NS
	Yes	9 (39.1%)	3 (30.0%)			
Nausea or vomiting	No	11 (47.8%)	6 (60.0%)	0.414[a]	0.520	NS
	Yes	12 (52.2%)	4 (40.0%)			
Diarrhea	No	12 (52.2%)	9 (90.0%)	4.309[a]	0.038	S
	Yes	11 (47.8%)	1 (10.0%)			
Abdominal pain	No	12 (52.2%)	9 (90.0%)	4.309[a]	0.038	S
	Yes	11 (47.8%)	1 (10.0%)			
Sore throat	No	14 (60.9%)	7 (70.0%)	0.251[a]	0.616	NS
	Yes	9 (39.1%)	3 (30.0%)			
Cough	No	14 (60.9%)	5 (50.0%)	0.337[a]	0.561	NS
	Yes	9 (39.1%)	5 (50.0%)			
Dyspnea	No	12 (52.2%)	3 (30.0%)	1.382[a]	0.240	NS
	Yes	11 (47.8%)	7 (70.0%)			
Diabetes	No	19 (82.6%)	4 (40.0%)	5.991[a]	0.014	S
	Yes	4 (17.4%)	6 (60.0%)			
Hypertension	No	22 (95.7%)	5 (50.0%)	9.764[a]	0.002	HS
	Yes	1 (4.3%)	5 (50.0%)			
IHD	No	23 (100.0%)	5 (50.0%)	13.554[a]	0.000	HS
	Yes	0 (0.0%)	5 (50.0%)			
Chest diseases	No	23 (100.0%)	6 (60.0%)	10.469[a]	0.001	HS
	Yes	0 (0.0%)	4 (40.0%)			
Duration of symptoms (days)	Median (IQR)	3 (3–4)	4 (3–7)	−1.465[c]	0.143	NS
	Range	2–14	1–11			
Need for mechanical ventilation	No	22 (95.7%)	8 (80.0%)	2.066[a]	0.151	NS
	Yes	1 (4.3%)	2 (20.0%)			
Ward/ICU	No	21 (91.3%)	7 (70.0%)	2.461[a]	0.117	NS
	ICU	2 (8.7%)	3 (30.0%)			
Severity classification	Mild	11 (47.8%)	3 (30.0%)	2.630[a]	0.269	NS
	Moderate to Severe	10 (43.5%)	4 (40.0%)			
	Critical	2 (8.7%)	3 (30.0%)			
HRCT findings	< 50% > 50%	22 (95.7%) 1 (4.3%)	6 (60.0%) 4 (40.0%)	6.891[a]	0.009	HS

P value > 0.05 non-significant (NS); *P value < 0.05* significant (S); *P value < 0.01* highly significant (HS), *ICU* intensive care unit, *HRCT* high-resolution computed tomography, *IHD* ischemic heart disease, *IQR* Interquartile range, *SD* Standard deviation

[a] Chi-square test

[b] Independent *t*-test

[c] Mann Whitney test

Table 6 Laboratory data in the study group according to mutation of the MTHFR gene

		MTHFR gene mutation		Test value	*P* value	Sig
		Absent	Present			
		No. = 23	No. = 10			
Laboratory parameters						
Serum IL-6 (ng/L)	Median (IQR)	26 (19–41)	42.2 (15–52)	−1.196[b]	0.232	NS
	Range	3–76	6–126			
CRP (mg/L)	Median (IQR)	16 (3.2–23)	16 (14–23)	−0.569[b]	0.569	NS
	Range	0.16–116	0.73–44			
Albumin (g/dL)	Mean ± SD	3.74 ± 0.24	3.33 ± 0.47	3.337[a]	0.002	HS
	Range	2.90–4.00	2.60–3.80			
Ferritin (ng/ml)	Median (IQR)	332 (121.6–443)	499 (345–544)	−2.157[b]	0.031	S
	Range	6–1007	77.5–2000			
D dimer (mg/l)	Median (IQR)	0.65 (0.45–0.77)	0.71 (0.56–1.1)	−1.689[b]	0.091	NS
	Range	0.08–1.1	0.55–2.1			
TLC (10^3/μL)	Mean ± SD	6.13 ± 2.15	7.04 ± 2.68	−1.036[a]	0.308	NS
	Range	3–12.3	4.2–10.5			
Lymphocytes (10^3/μL)	Mean ± SD	1.40 ± 0.58	1.12 ± 0.36	1.425[a]	0.164	NS
	Range	0.9–2.8	0.6–2			

P value > 0.05 non-significant (NS); P value < 0.05 significant (S); *P value < 0.01* highly significant (HS), *TLC* Total leucocytic count, *CRP* C-reactive protein

[a] Independent *t*-test

[b] Mann Whitney test

Table 7 Outcome data in the study group according to MTHFR gene mutation

[*n* (%)]		MTHFR gene mutation		Test value	*P*-value	Sig
		Absent	Present			
		No. = 23	No. = 10			
Outcome [*n* (%)]						
Cured/discharged	No	0 (0.0%)	3 (100.0%)	5.000[a]	0.025	S
	Yes	2 (100.0%)	0 (0.0%)			
Continued hospitalization	No	2 (100.0%)	2 (66.7%)	0.833[a]	0.361	NS
	Yes	0 (0.0%)	1 (33.3%)			
Death	No	23 (100.0%)	8 (80.0%)	4.897[a]	0.027	S
	Yes	0 (0.0%)	2 (20.0%)			
Radiological evidence of thrombosis	No	23 (100.0%)	7 (70.0%)	7.590[a]	0.022	S
	PE	0 (0.0%)	2 (20.0%)			
	DVT	0 (0.0%)	1 (10.0%)			
Duration of hospital admission	Median (IQR)	15 (14–16)	21 (9–75)	0.000[b]	1.000	NS
	Range	14–16	6–120			

P value > 0.05 non-significant (NS); *P value < 0.05* significant (S); *P value < 0.01* highly significant (HS)

[a] Chi-square test

[b] Mann–Whitney test

There are undoubtedly other factors contributing to COVID-19 patients' high risk of thrombosis; nevertheless, the available information is sparse and requires more research. Investigating the role of MTHFR gene mutation in the COVID-19 course in this context seems interesting [22].

In the study group, 14 patients had mild COVID-19, 14 patients had moderate COVID-19, and 5 patients

Table 8 MTHFR gene mutation risk factors in the study group

Parameter	AUC	Cut of point	Sensitivity	Specificity	PPV	NPV
Age	0.763	> 55	60.0	100.0	100.0	85.2
Albumin	0.759	≤ 3.2	50.0	95.65	83.3	81.5
Ferritin	0.739	> 453	70.0	82.61	63.6	86.4

AUC Area under the curve, *PPV* Positive predictive value, *NPV* Negative predictive value

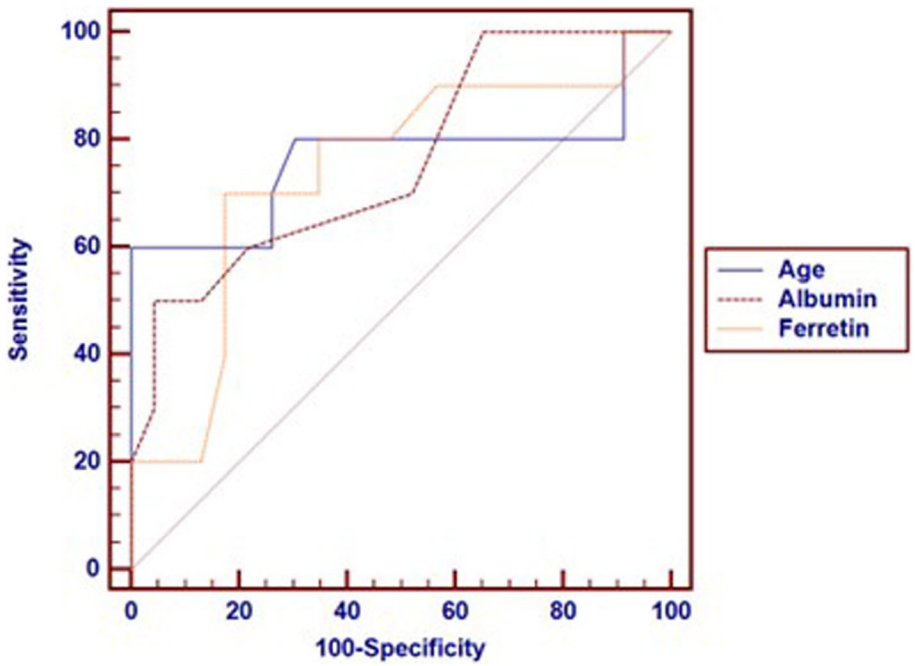

Fig. 1 ROC curve of age, albumin and ferritin as predictors of MTHFR gene mutation

had critical COVID-19, where 10 patients (33.3%) had MTHFR gene mutation.

By utilizing two separate groups, we found a significant number of patients in a study group (10 patients,33.3%) with MTHFR gene mutation who suffered from COVID-19 (9 patients with Heterozygous MTHFR gene mutation, 1 patient with Homozygous MTHFR gene mutation), with mild COVID-19 in three patients (DVT was detected in one of them), moderate COVID-19 in four patients (PE was detected in one of them), and critical degree of COVID-19 in three patients (one of them developed PE, while two died with sepsis). These match the correlation between poor prognosis of COVID-19 with MTHFR mutation and increased risk that was previously mentioned [23].

Five patients with MTHFR mutation had IHD (2 of them died, while 2 patients developed PE). However, more firm conclusions cannot be drawn because of the limited sample size and the low incidence of thrombotic events.

Among our cohort, at a significance level (*P* value) of 0.05, the expected statistical power to identify a significant difference in the rate of vascular events (5%) between the two groups is low (30%). It is noteworthy, however, that only one of the two patients who had a thrombotic event had a homozygous MTHFR gene mutation, while the other two had heterozygous MTHFR gene mutation, as determined by PCR. This agreed with research by Ponti et al. who discovered a significant correlation between the incidence and mortality of COVID-19 and the prevalence of homozygous MTHFR677 mutation. They also came to the conclusion that MTHFR C677T genetic polymorphism may affect COVID-19 incidence and severity. This data raises the possibility that COVID-19 patients with MTHFR gene mutations may have a higher thrombosis risk. However, additional research looking for MTHFR gene mutations in larger cohorts would significantly boost the statistical power to support this claim [24].

In the same context as our discovery that MTHFR-positive patients exhibit statistically significant radiological evidence of severe disease in their HRCT chest, according to a theory put forth by Karst et al., the MFTHR C677T polymorphism causes hyperhomocysteinemia, which in turn causes a severe course of COVID-19 [9].

In the end, it is noteworthy that the majority of MTHFR gene mutation patients (7 patients, 70%) did not experience symptomatic thrombotic events throughout COVID-19, including one subject with a history of IHD (of the other four IHD patients, two experienced PE, while the other two died). Stronger evidence is needed, but it is possible that the fact that these patients were in the early stages of the disease or were receiving early anticoagulation was the reason why the hypercoagulable state that caused the previously mentioned vascular events did not facilitate the development of new thrombotic episodes during SARS-CoV-2 infection. In agreement with this, a prior study indicates that long-term anticoagulation at admission might prevent thrombosis in COVID-19 patients [25]. One argument against routinely testing for MTHFR gene mutations could be the financial burden.

There is insufficient evidence to say if vitamin B and folic acid supplements will lower the cardiovascular risks linked to hyper-homocysteinemia or MTHFR genetic status if administered by MTHFR-positive individuals [16].

Limitations of the study

First, only a few patients were included in this single-center trial, which might reduce the applicability of findings to other populations. Second, the study did not target the relationship between the MTHFR gene mutation and other conditions that increase the risk of thromboembolism or affect outcomes in COVID-19 for example obesity and ischemic heart disease.

Conclusion

The findings of this pilot study, the first to examine the role of the MTHFR gene mutation in COVID-19 in Egypt, the incidence of MTHFR gene mutation was 30.3% of COVID-19 patients. Results suggest a potential association between inherited MTHFR gene mutation and severe form of COVID-19, thromboembolic events, and mortality. This connection should stimulate more investigation into the biology, and clinical implications of inherited MTHFR gene mutation in COVID-19, and the most effective management of anticoagulation in these patients.

Abbreviations

AUC	Area under the curve
COVID-19	Coronavirus disease of 2019
CRP	C-reactive protein
DVT	Deep venous thrombosis
EDTA	Ethylenediaminetetraacetic acid
FMASU	Faculty of Medicine–Ain Shams University
HRCT	High-resolution computed tomography
ICU	Intensive care unit
IHD	Ischemic heart disease
IL-6	Interleukin 6
IQR	Interquartile range
LDH	Lactate hydrogenase
MOHP	Ministry of health and population
MTHFR	Methylenetetrahydrofolate reductase
NPV	Negative predictive value
OR	Odds ratio
PE	Pulmonary embolism
PPV	Positive predictive value,
ROC curve	Receiver operating characteristic curve
RT-PCR	Real-time polymerase chain reaction
SARS COV2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SpO2 (%)	Peripheral oxygen saturation
TLC	Total leucocytic count

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Declarations

Ethics approval and consent to participate
After the study's protocol was revised and patients gave their written agreement, the ethics committee for scientific research at the Faculty of Medicine at Ain Shams University granted approval (the committee's reference ID number is FMASU R 130/2022).
-ClinicalTrials.gov ID: NCT05679414. https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S000CU2V&selectaction=Edit&uid=U00056R5&ts=2&cx=lrrb7q. Retrospectively registered. 9th January 2023.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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