Hypercoagulability in different respiratory diseases

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Received 11 January 2013; accepted 9 June 2013
Available online 3 July 2013

KEYWORDS
D-dimer (DD); Soluble fibrin complex (SFC); Thrombin antithrombin complex (TAT); Fibrinogen. coagulopathy

Abstract  Background: The risk of venous thromboembolism (VTE) is equally high in medical patients admitted to the hospital and those treated in the surgery wards. Patients who are immobilized due to heart failure, severe respiratory disease, cerebrovascular stroke and cancer are at a high risk of venous thrombosis.

Aim of the work: The aim of the study was to assess the impact of different respiratory insults on blood coagulation for early detection and prevention of thrombosis to open the way for thromboprophylaxis in such cases.

Patients and methods: The study included 25 apparently normal healthy control subjects and 141 patients with different respiratory disorders. All patients were subjected to full medical history taking, full clinical examination, and radiological evaluation. Computed tomography (CT) pulmonary angiography was done for all cases and lower limb duplex ultrasonography for all patients, at day 1 and day 5 of admission. The following coagulation markers were evaluated for all patients at day 1, 2, 3, 4, and 5 of admission including: Soluble fibrin complex, D dimer, thrombin antithrombin complex, antithrombin, protein C, protein S, and fibrinogen.

Results: It was found that, the mean values of soluble fibrin complex, D dimer and thrombin antithrombin complex were higher in patients with positive duplex ultrasonography and positive CT angiography than patients with negative duplex ultrasonography and CT angiography and the difference was statistically highly significant. The higher mean values for soluble fibrin complex was on the third day of admission, while the higher mean values for D dimer, thrombin antithrombin
Introduction

Although numerous studies about hemostatic biomarkers have been performed in patients suspected of deep vein thrombosis (DVT) or pulmonary embolism (PE), no data are available on the level of such hemostatic markers in non selected medical inpatients without any suspicion of acute venous thrombosis [1]. Because they induce venous stasis and inflammatory state, most acute medical diseases are considered as being associated with an increased risk of venous thromboembolism (VTE) [2].

A prethrombotic state may be defined as a condition characterized by an imbalance in hemostasis with a tendency for hyper-coagulability, due to pathological activation of the enzymes of the coagulation cascade, but without clinical signs of thrombosis or evidence of fibrin deposition. Hypercoagulable states can be classified into two broad categories: congenital and acquired. The former are generally inherited normalities of hemostasis clearly identified, while acquired hypercoagulable states include clinical conditions associated with an increased risk of thrombosis or evidence of fibrin deposition. Hypercoagulable states which carry high risks for the development of venous thromboembolism.

Conclusions: Estimation of soluble fibrin complex, D dimer, thrombin antithrombin complex and fibrinogen may be useful for early identification of the prethrombotic state which may help to prevent the onset of thrombotic disorders and thereby improve the outcome of various respiratory diseases. Common respiratory disorders especially, COPD exacerbation, pneumonia, interstitial pulmonary fibrosis, and lung cancer are considered to be hypercoagulable states which carry high risks for the development of venous thromboembolism.

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Patients and methods

Study design

This study was carried out at chest department, respiratory ICU and clinical pathology department of Zagazig University Hospitals in the period from October 2010 to January 2012. This study was conducted on 166 subjects, who were classified into two groups:-

(I) Control group: included 25 apparently normal healthy persons, 15 males and 10 females, their ages ranged from 23 to 46 years old with a mean of 35.8 ± 6.76 years.

(II) Patient group: included 141 patients with different respiratory disorders, 37 males and 104 females, their ages ranged from 33 to 65 years old with a mean of 52.3 ± 7.9 years. Those patients were subdivided according to their pathological diagnosis into:-

- (21) patients with exacerbated bronchial asthma (16) patients non mechanically ventilated and five mechanically ventilated patients.
- (38) patients with exacerbated chronic obstructive pulmonary disease (COPD) patients (25) patients non mechanically ventilated and 13 mechanically ventilated patients.
- (26) patients with acute exacerbation of interstitial pulmonary fibrosis. (IPF) (22) patients non mechanically ventilated and four mechanically ventilated patients.
- (31) patients with pneumonia (23) patients non mechanically ventilated and eight mechanically ventilated patients.
- (15) patients with lung cancer and (10) patients with malignant mesothelioma.

Exclusion criteria

Patients who have had diabetes mellitus, hepatic disorder, deep vein thrombosis (DVT), and suspect pulmonary embolism
cases diagnosed by: – (clinical probability of well’s criteria, D dimer and duplex at time of admission), disseminated intravascular coagulation (DIC), acute myocardial infarction (AMI) and thrombotic thrombocytopenic purpura were excluded from the study as, fibrin-related markers such as D-dimer, and soluble fibrin complex (SFC) were reported to be elevated in such diseases [8].

Patients who have had anticoagulant therapy were also excluded from the study as the plasma levels of thrombin antithrombin complex (TAT), D-dimer, antithrombin (AT) and soluble fibrin complex were affected. [9].

Methods

All patients were subjected to the following:-

(1) Full medical history taking stressing on smoking habit, occupational exposure, drug intake, duration of illness and history of any other diseases.

(2) Full clinical examination

- General examination.
- Local chest examination.

(3) Radiological evaluation

(a) Plain chest films (postero-anterior and lateral views) (done for all cases).
(b) High resolution CT (22 cases).
(c) Conventional chest CT (35 cases) and CT guided biopsy when indicated (25 cases).

Percutaneous transthoracic fine needle aspiration biopsy (TTNA) was done under CT guidance from suspected peripheral pulmonary lesions by using spinal needle gauge 22 according to the method of Hideo and Nobuo [10].

(d) CT pulmonary angiography was done for all cases (141 cases).
(e) Lower limb duplex ultrasonography was done for all patients, at day 1, and at day 5 of admission.

Compression ultrasonography (Prisma Diasonics, Les Ulis, France) with superficial probe (7.5 MHz) was used for DVT assessment. This device currently incorporates color Doppler flow with a B-mode image of vein projected either longitudinally or transversely [11].

Technique of duplex ultrasonography

Compression ultrasound with venous imaging (real-time B-mode) was done utilizing a combination of gray scale duplex and color doppler imaging to evaluate the deep venous system of both lower limbs. The compression technique was used, beginning at the inguinal ligament, and the common femoral vein and great saphenous vein were evaluated, then the superficial femoral vein, which is a part of the deep venous system, was evaluated, the deep femoral vein was evaluated at the bifurcation of common femoral vein, then popliteal and calf veins were evaluated [12].

Color imaging was used to better evaluate the venous blood flow and this is especially useful for non-occlusive thrombi and for calf vein evaluation in obese patients.

Criteria for the diagnosis of acute DVT

- Non-compressibility of the vein, this is the most reliable sign of acute DVT.
- Echogenic thrombus within the vein lumen.
- Venous distention.
- Complete absence of color Doppler signal from the vein lumen [13]

(4) Pulmonary function tests

These tests were done for 41 cases, (COPD, asthma and IPF patients). These were carried out using a portable spirometer “Vitalograph copd-6™” apparatus. Expiratory flow-volume curve was performed for 41 subjects before and 20 min after inhalation of four puffs of salbutamol (400 μg) metered dose inhaler (MDI).

The following parameters were obtained:

- Forced expiratory volume in the first second (FEV1) as absolute value and percentage of the predicted value (FEV1%).
- FEV1%/FVC ratio.

(5) Arterial blood gas analysis

Using blood gas analyzer (ABL-330-Radiometer Copenhagen system) all values were obtained in room air except for mechanically ventilated patients.

(6) Thoracocentesis and pleural fluid examination

Thoracocentesis and pleural fluid examination were done for 17 cases, (10 cases of mesothelioma, five cases of pneumonia and two cases of bronchogenic carcinoma).

Pleural fluid examination included:

(a) Physical appearance.
(b) Protein level.
(c) Glucose level.
(d) LDH.
(e) Total and differential white blood cell count.
(f) Gram stain.
(g) Z.N. staining.
(h) Culture and sensitivity for aerobic and anaerobic organisms.
(i) Culture for acid fast bacilli.
(j) Cytological examination.

(7) Blind pleural biopsy by Abram’s needle

It was done for seven cases of mesothelioma according to the method of James et al. [14].

(8) Fiberoptic bronchoscopy

Fiberoptic bronchoscope was done for 15 cases [15].
It was done using prototype (PENTAX FB-19TV, Japan) and the following procedures were performed via bronchoscope

- Bronchoalveolar lavage (BAL).
- Endobronchial biopsy.
- Bronchial washing.
- Bronchial brushing.
- Post bronchoscopic sputum cytology.
Estimation of soluble fibrin monomer complex (SFMC ELISA kits, MyBioSource co., CA, USA).

Principle of the method

The microtiter plate provided in this kit has been pre-coated with an antibody specific to SFMC. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for SFMC and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain SFMC, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of SFMC in the samples is then determined by comparing the O.D. of the standards to the standard curve. Cut off value: 6.25 µg/ml.

Estimation of D-dimer (VIDAS® D-Dimer Exclusion™ (DD2))

Principle of the method

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase with an anti-fibrin degradation product (FbDP) monoclonal antibody adsorbed on its surface as well as the pipetting device for the assay. The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a “sandwich”. In the second step, the remaining free antigen sites are saturated by cycling the conjugate in the fifth well of the strip in and out of the SPR. Unbound components are eliminated during the washing steps. Cut off value: 500 ng/ml.

Estimation of thrombin antithrombin (TAT) (IMUBIND® TAT ELISA assay TEST)

Principle of the method

Diluted plasma samples are added to microwells coated with a monoclonal antibody against thrombin. During an incubation period, TAT complexes present in the sample will bind to the antibody coated to the wells. Following a washing step, a biotinylated monoclonal antithrombin III antibody is added to the microwells and binds to the TAT complexes captured on the plate during a short incubation period. A streptavidinhorseradish peroxidase conjugate (SA-HRP) is added to the microwells to complete the formation of the antibody-enzyme detection complex. Following another washing step, the addition of a perborate-3,3,-5,5-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance a standard curve determines the level of TAT in the diluted plasma sample. Expected values: 1.2–5.3 ng/ml.

Estimation of antithrombin (AT) Activity (COAMATIC® Antithrombin, Chromo-genix, Molndall, Sweden)

Summary and principle of the test

Antithrombin is the most important natural inhibitor of the coagulation cascade. By inhibiting the coagulation proteases, especially thrombin, factor Xa, and factor IXa, AT prevents uncontrolled coagulation and thrombosis. Plasma is incubated with an excess of factor Xa (FXa) in the presence of heparin. The residual quantity of FXa is determined by the rate of hydrolysis of the chromogenic substrate S-2765. The pNA release measured at 405 nm is inversely proportional to the AT level in Expected values: >70% in a normal healthy population.

Estimation of protein C (Corgenix, REAADS Protein C Antigen Test)

Principle of the test

The protein C Antigen assay is a sandwich ELISA. A capture antibody specific for human protein C is coated to 96-microwell polystyrene plates. Diluted patient plasma is incubated in the wells, allowing any available protein C to bind to the antihuman protein C antibody on the microwell surface. Bound protein C is quantitated using horseradish peroxidase (HRP) conjugated anti-human Protein C detection antibody. Following incubation, unbound conjugate is removed by washing. A chromogenic substrate of (TMB) and hydrogen peroxide (H_2O_2) is added to develop a colored reaction. The intensity of the color is measured in optical density (O.D.) units with a spectrophotometer at 450 nm. Protein C antigen relative percent concentrations in patient plasma are determined. Expected Value: The normal range when normal plasma samples were tested by REAADS Protein C Antigen assay was 72–160%.
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Estimation of protein S (Corgenix, READS protein S Antigen Test kits)

Principle of the test

The Monoclonal Free Protein S Antigen assay is a sandwich ELISA. A capture monoclonal antibody specific for human Free Protein S is coated to 96-microwell polystyrene plates. Diluted patient plasma is incubated in the wells, allowing any available Free Protein S to bind to the anti-human Free protein S monoclonal antibody on the microwell surface. Bound Free Protein S is quantitated using horseradish peroxidase (HRP) conjugated polyclonal anti-human Protein S detection antibody. A chromogenic substrate of tetramethyl-benzidine (TMB) and hydrogen peroxide (H₂O₂) is added to develop a colored reaction. The intensity of the color is measured in optical density (O.D.) units with a spectrophotometer at 450 nm. Expected values. The normal range for Free Protein S for this assay is 50–150%.

Estimation of fibrinogen (Clauss assay method, Immunospec® Corporation)

Principle

Immunospec Fibrinogen test kit is based on the Clauss method of quantifying plasma fibrinogen. The Clauss method measures the rate of fibrinogen fibrin conversion in the presence of excess thrombin and has been shown to be rapid, sensitive and precise. When diluted plasma is clotted with excess thrombin, the fibrinogen level is inversely proportional to the clotting time. A calibration curve is prepared from a fibrinogen reference and plotted on log–log paper. Expected value range level in human plasma is considered to be 150–400 mg/dl.

Statistical analysis

The demographic, clinical, radiological, physiological and pathological data gathered together with the patients’ outcome were tabulated and statistically analyzed and coded, entered and checked to an Epi-info file using Epi-info version 10 computer packages. Data were summarized using; the arithmetic mean as an average describing the central tendency of observations, the standard deviation (SD) as a measure of dispersion of the results around the mean, the number of observations for each variable studied (NO). Comparison of means: ANOVA and multiple comparison tests (LSD and paired t-test) were used. For all the above-mentioned statistical tests, the threshold of significance is fixed at the 5% level (p-value), a p-value ≥ 0.05 indicates non-significant results, a p-value < 0.05 indicates significant results, a p-value < 0.01 indicates high significant results, and a p-value < 0.001 indicates very high significant results.

Results and discussion

The vast majority (80%) of hospitalized patients with symptomatic venous thromboembolism (VTE) has not undergone recent surgery. Furthermore, 70–80% of cases of fatal pulmonary embolism (PE) in the hospital occur in medical (non-surgical) patients. [16]. So, the aim of this study was to assess the impact of different respiratory insults on blood coagulation.

This study was conducted on 141 patients with different respiratory disorders, 81 males and 60 females, their ages ranged from 33 to 65 years old with a mean of 52.3 ± 7.9 years. Twenty-five apparently healthy control subjects, 15 males and 10 females, their ages ranged from 23 to 46 years old with a mean of 35.8 ± 6.76 years were also included in our study (Table 1).

In the current study, the mean values of SFC, DD and TAT were higher in patients with positive duplex than negative duplex and this difference was statistically highly significant while, there was no statistically significant difference in the mean values of other coagulation markers between patients with positive duplex and negative duplex patients (Table 3). Also, in the current study, the mean values of SFC, DD and TAT were higher in positive CT angiography than negative CT angiography and the difference was statistically highly significant while there was no statistically significant

### Table 1 Demographic data of all studied subjects.

| Age (years) | Patients group n = 141 | Control group n = 25 |
|-------------|------------------------|----------------------|
| Mean ± SD (range) | 52.3 ± 7.9 (33–65) | 35.8 ± 6.76 (23–46) |
| Gender      | No         | %        | No         | %        |
| Male        | 81         | 57.4     | 15         | 60       |
| Female      | 60         | 42.6     | 10         | 40       |
| Smoking    |            |          |            |          |
| Non-smokers | 50         | 35.4     | 14         | 56       |
| Mild-smokers | 6         | 4.3      | 2          | 8        |
| Moderate-smokers | 39       | 27.7     | 5          | 20       |
| Heavy-smokers | 46       | 32.6     | 4          | 16       |

This table shows that 57.4% of studied patients were males, while 42.6% were females. Non smokers were 35.4%, heavy smokers 32.6%, moderate smokers 27.7% and 4.3% mild smokers. The mean age of studied patients was 52.3 ± 7.9 years and their age range was 33–65 years. In the control group 60% of studied subjects were males and 40% were females. Non smokers were 36%, heavy smokers 16%, moderate smokers 20% and 8% mild smokers. The mean age of control subjects was 35.8 ± 6.76 years and their age range was 23–46 years.
difference in mean values of other coagulation markers between patients with positive and negative CT angiography patients (Table 4).

In agreement with these results, Tsuji et al. [17] found that the plasma levels of SFC and D-dimer were significantly higher in patients with VTE than patients without VTE. Soluble fibrin complex and D-dimer are considered as markers of hyper-coagulable state, as both parameters are reported to be elevated in DVT [18]. Also LaCapra et al. [19], concluded that, TAT is markedly increased in patients with acute DVT or PE. As well as, Garcia-Avello et al. [20] studied patients with acute or subacute lower limb arterial thrombosis and found that, there was an elevation of TAT, fibrin monomers and D-dimer levels. In addition to that, Ota et al. [21] studied suspect patients to have DVT, about 10% of patients were diagnosed with DVT. He found that, plasma levels of D-dimer and SFC in that percentage of patients in whom DVT were confirmed were significantly higher than of those without DVT.

In the present study, the higher mean values for soluble fibrin complex (SFC) were on the third day, while the higher mean values for both D dimer (DD), thrombin antithrombin complex (TAT) were on the fifth day, however for antithrombin (AT), protein C and protein S, there were no detectable variations in their levels on all 5 days (Table 5).

In agreement with these results Nieuwenhuizen et al. [22] reported that, the plasma levels of SFC were significantly elevated in patients with thrombosis within a week before its onset, but the plasma levels of D-dimer, and thrombin antithrombin complex (TAT) were not significantly increased within a week before the onset of thrombosis, thus indicating that the SFC assay may otherwise be useful for detecting a prethrombotic state and the diagnosis of the prethrombotic state. With regard to the changes in the levels of D-dimer and SFC, the plasma levels of D-dimer were further increased after the onset of DVT, and they were still high at 7 days after its onset, whereas the plasma SFC levels diminished relatively soon after development of thrombosis. These findings suggest that the SFC reflects the early phase of DVT/PE whereas D-dimer reflects the secondary fibrinolysis after clot formation. The plasma SFC level will decrease 2–3 days after onset of thrombosis, but the D-dimer level will not increase immediately after its onset [43].

| Table 2 | Presentation of different diseases in the studied patients. |
|---------|---------------------------------------------------------|
| Disease | Frequency No | %            |
| COPD    | 38           | 27           |
| Bronchial asthma | 21 | 14.9       |
| Interstitial pulmonary fibrosis (IPF) | 26 | 18.5       |
| Pneumonia | 31           | 21.9         |
| Malignancy | 25           | 17.7         |
| Lung cancer | 15           | 60           |
| Malignant mesothelioma | 10 | 40          |

This table shows that COPD patients presented 27% of the studied patients, while bronchial asthma patients were 14.9%, IPF cases were 18.5%, pneumonia cases were 21.9% and 17.7% were malignant diseases (60% lung cancer cases and 40% malignant mesothelioma cases).

| Table 3 | Levels of different coagulation markers regarding lower limb duplex U.S. results in the studied patients. |
|---------|--------------------------------------------------------------------------------------------------|
| Values of coagulation markers (Mean ± SD) Positive duplex U.S. (No. 33 cases) Negative Duplex U.S. (No. 108 cases) t p |
| Soluble fibrin complex (SFC) | 286.4 ± 21.4 | 147.6 ± 86.3 | 9.12 <0.001 |
| D dimmer (DD) | 29.5 ± 11.3 | 11 ± 19.1 | 91.61 <0.001 |
| Thrombin antithrombin complex (TAT) | 17.5 ± 3.8 | 11.2 ± 5.7 | 5.93 <0.001 |
| Antithrombin (AT) | 93.5 ± 11.3 | 99.4 ± 13.9 | 2.23 >0.05 |
| Protein C | 116 ± 15.6 | 112.6 ± 14.5 | 1.62 >0.05 |
| Protein S | 115.5 ± 17.3 | 112.7 ± 19.1 | 0.96 >0.05 |
| Fibrinogen | 350.1 ± 44 | 326.7 ± 62 | 4.23 >0.05 |

This table shows that the mean values of SFC, DD and TAT were higher in patients with positive duplex than negative duplex and this difference was statistically highly significant while there is no statistically significant difference in the mean values of other coagulation markers between both groups.

| Table 4 | Levels of different coagulation markers in all patients regarding CT angiography results. |
|---------|----------------------------------------------------------------------------------|
| Values of coagulation markers (Mean ± SD) Positive CT angiography (No. 17 cases) Negative CT angiography (No. 124 cases) t p |
| Soluble fibrin complex (SFC) | 292.7 ± 22.4 | 164.6 ± 92.2 | 5.68 <0.001 |
| D dimmer (DD) | 715.8 ± 176.4 | 352.8 ± 151.6 | 11.57 <0.001 |
| Thrombin antithrombin complex (TAT) | 16.8 ± 4.2 | 12.1 ± 5.9 | 3.18 <0.001 |
| Antithrombin (AT) | 98.4 ± 13.5 | 95 ± 13.6 | 0.98 >0.05 |
| Protein C | 117.99 ± 15.2 | 110.8 ± 14 | 1.81 >0.05 |
| Protein S | 115.1 ± 17.1 | 111.4 ± 12.4 | 0.85 >0.05 |
| Fibrinogen | 347.1 ± 57.7 | 321.4 ± 60 | 4.63 >0.05 |

This table shows that the mean values of SFC, DD and TAT were higher in patients having positive CT angiography result than patients with negative results and the difference was statistically highly significant while there was no statistically significant difference in mean values of other coagulation markers between both groups.
The concentration of soluble fibrin complex did not change significantly during the initial stage after surgery (1–2 h), but it increased significantly thereafter until day 3, and then decreased gradually reaching a plateau thereafter. On the contrary, the D-dimer increased only slightly after surgery and remained at low levels during the first 3 days, but it became significantly increased from days 5 to 10. The level of D dimer remained higher than the preoperative level even on day 14.

In the present work, there was a highly statistically significant difference in soluble fibrin complex, D-dimer and thrombin–antithrombin complex, fibrinogen levels between all disease groups and the control subjects. The levels of those markers in bronchial asthma patients were lower than in other respiratory diseases and control subjects. The levels of such markers in bronchial asthma patients were lower than other respiratory diseases and control subjects. The levels of those markers in bronchial asthma patients were lower than other respiratory diseases and control subjects. The levels of those markers in bronchial asthma patients were lower than other respiratory diseases and control subjects. The levels of those markers in bronchial asthma patients were lower than other respiratory diseases and control subjects.

In agreement with the results of this study, Shin [18] found that, plasma levels of SFC, a marker of thrombin generation, D-dimer, a marker of in vivo thrombin and plasmin activation, and fibrinogen when measured in COPD patients compared to controls matched for sex and age were significantly higher in COPD patients than in healthy subjects. Near similar results were mentioned by Anetta Undas et al. [27] who studied COPD patients compared with controls matched for age, sex, weight, and smoking and found that antithrombin, protein C and protein S did not differ between both groups. The plasma fibrinogen level was studied in patients with chronic obstructive pulmonary disease and control group, the plasma fibrinogen level was apparently higher in exacerbated patients than in control group. The elevated plasma fibrinogen level may be associated with the hypercoagulability state of chronic obstructive pulmonary disease and contributes to thrombi in small pulmonary arteries and arterioles [28].

Psuja et al. [29] found that in severe infection including community acquired pneumonia, specific markers of plasma hypercoagulability, that is, thrombin–antithrombin (TAT) complexes, prothrombin activating factor F 1 + 2, SFC and D dimer were all markedly increased. Gouin-Thibault et al. [30] evaluated the thrombophilic state of different cancer patients including lung cancer patients, it was found that there was elevation of clotting factors, such as, fibrinogen/ fibrin degradation products, hyperfibrinogenemia and thrombocytosis and elevation of specific markers of activation of coagulation such as: fibrinopeptide A, soluble fibrin fragment 1 + 2, thrombin–antithrombin complexes and D dimers. [22].

### Table 5
Comparison between the mean values of different coagulation markers on the first 5 days of admission of all studied patients.

| Values of coagulation markers (Mean ± SD) | Day 1       | Day 2       | Day 3       | Day 4       | Day 5       |
|-----------------------------------------|-------------|-------------|-------------|-------------|-------------|
| Soluble fibrin complex (SFC)            | 127.5 ± 65  | 159.5 ± 83.4| 180.1 ± 96.3| 145.5 ± 74.3| 135.7 ± 69  |
| D-dimer                                 | 255.2 ± 113 | 266.2 ± 9.6 | 282.2 ± 122 | 319.5 ± 129.9| 437.6 ± 220 |
| Thrombin–antithrombin (TAT)             | 6.4 ± 2.6   | 8 ± 3.4     | 9.4 ± 3.9   | 10.9 ± 4.6   | 12.7 ± 5.9  |
| Protein C                               | 117.9 ± 15.2| 105.9 ± 17.9| 115.9 ± 11.2| 111.9 ± 13.4| 109.7 ± 10.7|
| Protein S                               | 114.1 ± 16.6| 115.8 ± 17.2| 113.8 ± 11.2| 118.8 ± 11.4| 116.8 ± 10.2|
| Anti-thrombin(AT)                       | 103.5 ± 13.3| 98 ± 13.5   | 94 ± 10.4   | 100 ± 13.5   | 99 ± 10.9   |
| Fibrinogen                              | 296 ± 10.8  | 298 ± 11    | 299 ± 12    | 300.1 ± 59   | 324.5 ± 60.4|

### Table 6
Comparison between the levels of coagulation markers among different respiratory diseases.

| Values of coagulation markers (Mean ± SD) | COPD          | Asthma        | IPF           | Malignancy    | Pneumonia     | Control      | p         |
|-----------------------------------------|---------------|---------------|---------------|---------------|---------------|--------------|-----------|
| Soluble fibrin complex (SFC)            | 179.4 ± 73    | 6 ± 0.9       | 201.7 ± 65.3  | 232.1 ± 46.3  | 218 ± 75      | 3.1 ± 0.34*  | <0.001    |
| D dimer                                 | 444 ± 225     | 354.7 ± 133   | 396.5 ± 206   | 503.8 ± 199   | 475 ± 238     | *371 ± 72.7  | >0.001    |
| Thrombin antithrombin complex           | 14.3 ± 4.0    | 1.7 ± 0.8     | 14.5 ± 4.3    | 14.5 ± 3.6    | 15.17 ± 4.4   | *3.6 ± 0.4   | >0.001    |
| Fibrinogen                              | 338.6 ± 45    | 257.6 ± 76    | 335.7 ± 60    | 336.7 ± 40.4  | 333.4 ± 49    | *151.6 ± 4.5 | >0.001    |
| Anti-thrombin                           | 101.6 ± 13.3  | 98 ± 14.3     | 97.1 ± 13.9   | 98.2 ± 13.9   | 93.8 ± 13.5   | 100.8 ± 21.1 | >0.05     |
| Protein C                               | 108 ± 17.5    | 115.4 ± 14    | 112.7 ± 21    | 101 ± 12      | 95 ± 16       | 112 ± 14.4   | >0.05     |
| Protein S                               | 118.8 ± 20.2  | 109 ± 16.4    | 118 ± 16.9    | 113.6 ± 12.4  | 116 ± 16.5    | 116 ± 16.5   | >0.05     |

This table shows that, there was highly statistically significant difference in SFC, D dimer, TAT and fibrinogen levels between all disease groups and control subjects. The levels of such markers in bronchial asthma patients were lower than other respiratory diseases and control subjects and this difference was highly statistically significant. There was no statistically significant difference between different diseases groups and control subjects regarding the levels of AT, protein C and protein S.
Kubo et al. [31] found that patients with IPF in acute exacerbation had elevated plasma level of D-dimer, and the plasma D-dimer levels in patients who died from acute exacerbations of IPF were significantly higher than those in survivors during acute exacerbation of IPF.

Jose et al. [7] studied patients with CAP and found that there is elevated D-dimer level in those patients and that elevated D-dimer levels were associated with radiologic pneumonia extension and poor outcome.

Patients with lung cancer have a higher risk of developing venous thromboembolism. D-dimer level significantly was elevated in those patients. There were no significant differences between males and females. High plasma D-dimer concentrations turned out to be a strong predictor of poor outcome [32].

Suzan Salama et al. [5] concluded that, thrombin antithrombin (TAT), which indicates activation of coagulation system increased significantly in COPD patients with PE than in those without PE. Moreover, the frequency of PE increased significantly as the TAT level increases. Eric et al. [8] measured plasma coagulation markers (D-dimer, antithrombin, factor IX, and thrombin–antithrombin complex [TAT]) at the time of presentation of CAP patients to the emergency department and daily during the first week of hospitalization. Coagulation abnormalities were common, especially for D-dimer and TAT and concluded that, coagulation abnormalities were common and persistent in CAP patients. Ferrigno and Bucchen [33] had found several laboratory abnormalities including prolonged and shortened PT and PTT, increased fibrinogen, fibrin degradation products, TAT, and thrombocytosis in patients with lung cancer. Wedzicha et al. [34] found that, the mean values of plasma fibrinogen were elevated in 67 patients of 93 COPD patients with exacerbations. Li Run-ying et al. [35] concluded that, in complicated pneumonia, the values of D-dimer, fibrinogen increased, and AT decreased gradually. There are

**Table 7** Comparison between the levels of coagulation markers in moderate and severe COPD patients.

| Values of coagulation markers (Mean ± SD) | Severe COPD (15 patients) | Moderate COPD (10 patients) | t   | p   |
|-----------------------------------------|---------------------------|----------------------------|-----|-----|
| Soluble fibrin complex                   | 231.3 ± 5                 | 101.7 ± 34                 | 6.85| <0.001|
| D dimer                                 | 307.2 ± 85.9              | 198 ± 70                   | 3.34| <0.001|
| Thrombin antithrombin complex           | 17.3 ± 2.6                | 9.2 ± 1                    | 9.4 | <0.001|
| Antithrombin                            | 93.7 ± 12.9               | 103 ± 10.6                 | 1.89| >0.05 |
| Protein C                               | 113.3 ± 16.3              | 130 ± 14.7                 | 1.55| >0.05 |
| Protein S                               | 115.7 ± 19.8              | 123.5 ± 20.9               | 0.94| >0.05 |
| Fibrinogen                              | 361.5 ± 41                | 297.7 ± 37                 | 3.9 | >0.001|

This table shows that the mean values of SFC, DD, TAT and fibrinogen were higher in severe COPD patients than in moderate COPD patients and the difference was statistically significant, while in AT, Protein C and Protein S there was no statistically significant difference.

**Table 8** Comparison between the levels of coagulation markers in moderate and severe bronchial asthma patients.

| Values of coagulation markers (Mean ± SD) | Severe asthma (8 patients) | Moderate asthma (8 patients) | t   | p   |
|------------------------------------------|---------------------------|----------------------------|-----|-----|
| Soluble fibrin complex                   | 6.4 ± 0.6                 | 5.8 ± 1.1                  | 0.9 | >0.05 |
| D dimer                                  | 387.8 ± 144               | 263.5 ± 110                | 1.93| >0.05 |
| Thrombin antithrombin                    | 1.44 ± 0.8                | 1.3 ± 0.76                 | 0.97| >0.05 |
| Antithrombin                             | 99.4 ± 16                 | 105.1 ± 9.3                | 0.86| >0.05 |
| Protein C                                | 113.7 ± 15.5              | 117.1 ± 14.9               | 0.4 | >0.05 |
| Protein S                                | 122.5 ± 20.7              | 115 ± 17.5                 | 0.78| >0.05 |
| Fibrinogen                               | 261 ± 62.9                | 206.4 ± 69                 | 1.65| >0.05 |

This table shows that there is no statistically significant difference in coagulation markers between moderate and severe asthmatic patients.

**Table 9** Mean values of coagulation markers in lung cancer and malignant mesothelioma cases.

| Values of coagulation markers (Mean ± SD) | Lung cancer (15 patients) | Malignant mesothelioma (10 patients) | t   | p   |
|------------------------------------------|---------------------------|--------------------------------------|-----|-----|
| Soluble fibrin complex                   | 230.1 ± 46.2              | 235.2 ± 48.7                        | 0.26| >0.05|
| D dimer                                  | 394 ± 120.7               | 352.8 ± 120                        | 0.83| >0.05|
| Thrombin antithrombin                    | 15.4 ± 3.1                | 13.1 ± 4                           | 1.66| >0.05|
| Antithrombin                             | 96 ± 13.2                 | 101.5 ± 15.3                      | 0.96| >0.05|
| Protein C                                | 115.7 ± 11.2              | 119.5 ± 17.1                      | 0.58| >0.05|
| Protein S                                | 111.3 ± 11.8              | 118.0 ± 14.2                      | 0.21| >0.05|
| Fibrinogen                               | 298.9 ± 44.6              | 314.8 ± 36.8                      | 0.93| >0.05|

This table shows that there is no significant statistical difference in the mean values of all coagulation markers between lung cancer and malignant mesothelioma cases.
**Table 10** Comparison of mean values of coagulation markers between mechanically and non mechanically ventilated patients among different respiratory diseases.

| Disease            | Non Mech.vent. Patients | Mechanicalvent. Patients |
|--------------------|-------------------------|--------------------------|
| **Protein C**      | 120.4 ± 11.4            | 115.7 ± 10.5             |
| **Protein S**      | 115.4 ± 13.9            | 106.5 ± 14.5             |
| **Protein F**      | 315.9 ± 50.3            | 225.7 ± 41.9             |
| **Fibrinogen**     | 311.8 ± 51.1            | 346.4 ± 26.8             |
| **Antithrombin**   | 110.7 ± 8.7             | 110.7 ± 6.7              |
| **D dimer**        | 514.1 ± 104             | 453.2 ± 69.9             |
| **SFC**            | 231 ± 42                | 246 ± 84                 |
| **Thrombin complex**| 14.6 ± 2.5              | 14.1 ± 4.5               |
| **Protein F**      | 315.9 ± 50.3            | 225.7 ± 41.9             |
| **Fibrinogen**     | 311.8 ± 51.1            | 346.4 ± 26.8             |
| **Antithrombin**   | 110.7 ± 8.7             | 110.7 ± 6.7              |
| **D dimer**        | 514.1 ± 104             | 453.2 ± 69.9             |
| **SFC**            | 231 ± 42                | 246 ± 84                 |
| **Thrombin complex**| 14.6 ± 2.5              | 14.1 ± 4.5               |
| **Protein F**      | 315.9 ± 50.3            | 225.7 ± 41.9             |
| **Fibrinogen**     | 311.8 ± 51.1            | 346.4 ± 26.8             |
| **Antithrombin**   | 110.7 ± 8.7             | 110.7 ± 6.7              |
| **D dimer**        | 514.1 ± 104             | 453.2 ± 69.9             |

This table shows that there were statistically significant differences in mean values of SFC and D dimer between mechanically ventilated COPD and pneumonitis patients, than non mechanically ventilated patients; it was found that there was elevation of clotting factors, such as, fibrinogen/fibrin degradation products, hyperfibrinogenemia.

The results of this study showed that, the mean values of coagulation markers SF, DD, TAT and fibrinogen were higher in sever COPD patients than in moderate COPD patients and the difference was of high statistical significance, while regarding the levels of AT, Protein S and Protein C there were no statistically significant difference (Tables 7 and 8).

Gonen et al. [36] found that the VTE increased with the increasing severity of COPD as it was higher in stage IV severity than other stages and also, with increased severity of exacerbation. Sheng-chen et al. [37] investigated patients with acute exacerbations of COPD in the respiratory intensive care unit and found that the DVT prevalence was 10.7% by ultrasonography. DVT and PE were more likely in the setting of severe decompensated COPD.

The results of the current work showed that, there was no statistically significant difference in the mean values of all coagulation markers between mesothelioma and bronchogenic carcinoma (Table 9). In agreement with these results Goldenberg et al. [38] stated that, there was no statistically significant difference in the level of coagulation markers between lung carcinoma and malignant mesothelioma.

Our results show that the mean values of SFC and D dimer were higher in both mechanically ventilated COPD and pneumonitis patients than non mechanically ventilated patients, while the difference was statistically not significant between mechanically ventilated and non mechanically ventilated patients with bronchial asthma, IPF and malignant patients (Table 10). In agreement with our results, Feng Yan et al. [39] found that the levels of D-dimer and fibrinogen were significantly increased in COPD ventilated group compared with non ventilated group. So, he concluded that, prethrombotic state exists in mechanically ventilated COPD patients and aggressive anticoagulation therapy, can shorten the time of weaning and reduce the mortality in these patients. Guinea et al. [40] showed that plasma D-dimer level is increased significantly in CAP group compared to control group. Also the D-dimer level increases proportionally with the severity of CAP including patients who were mechanically ventilated. This condition supports the relationship between D-dimer level and the severity of CAP.

**Conclusions**

Common respiratory disorders especially, COPD exacerbations, pneumonia, interstitial pulmonary fibrosis and lung cancer are associated with an increased risk of the development of venous thromboembolism.

Early detection of soluble fibrin complex, thrombin antithrombin complex, D dimer and fibrinogen may be useful for the identification of the prethrombotic state which may help to prevent the onset of thrombotic disorders such as deep venous thrombosis, pulmonary embolism and disseminated intravascular coagulation and thereby improve the outcome of various respiratory diseases.
Recommendations

Identifying medical patients with different respiratory diseases at risk of venous thromboembolism and providing effective anticoagulant prophylaxis is an important health care priority to reduce the burden of this morbid and sometimes fatal disease.

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