



FOUNTAIN JOURNAL OF NATURAL & APPLIED SCIENCES

A Publication of the College of Natural & Applied Sciences
Fountain University, Osogbo, Nigeria



Effects of gasoline exposure on some haematological and coagulation parameters among fuel attendants in Sokoto metropolis

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ABSTRACT

Gasoline is always available in the atmosphere whenever it is distributed, particularly at gas stations and depots. Excessive benzene exposure has been shown to affect bone marrow, leading to a reduction in circulating blood cells. This cross-sectional study aimed at determining the effects of petroleum on haematological and coagulation parameters among occupational workers. A total of 80 participants were recruited, 40 exposed (5 females, 35 males) and 40 controls (20 females, 20 males). A simple random sampling method was used, and a questionnaire was administered. Socio-demographic data of the study participants were taken. A full blood count was performed using a haematological analyser. Coagulation parameters were measured using the manual method at 37 °C. The results showed a statistically significant increase in the exposed group compared to the control group in Red Blood Cells (RBC) (5.77 ± 0.78 ; 5.77 ± 0.78), Haemoglobin (Hb) (13.27 ± 1.48 ; 11.81 ± 1.56), and Haematocrit (HCT) (41.89 ± 5.19 ; 38.02 ± 4.13), $p < 0.001$, respectively. The Partial Thrombin Time and Kaolin (PTTK) results showed a statistically significant increase in the exposed compared to the control group (41.13 ± 8.37 ; 30.28 ± 5.61), $p < 0.001$. The Prothrombin Time (PT) (13.03 ± 0.28 ; 14.25 ± 1.32) and International Normalised Ratio (INR) (1.002 ± 0.02 ; 1.096 ± 0.09) $p < 0.001$ were statistically decreased between the exposed and the control group, respectively. There was a statistically significant negative correlation between PT, RBC ($r = -0.295$), PT, HCT ($r = -0.310$), and between INR, RBC ($r = -0.301$), INR, HCT ($r = -0.315$), $p < 0.05$, respectively. This research showed a significant effect on the coagulation parameters. Hence, protective equipment for petrol station workers should be used to minimise exposure to gasoline.

ARTICLE INFO

Article history:

Received July 2025

Revised September 2025

Accepted September 2025

Keywords:

Petrol station workers, gasoline, haematological and coagulation parameter



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Introduction

Crude petroleum is used to distil gasoline, and due to its volatility, gasoline is always easily available in the atmosphere whenever it is distributed, particularly at gas stations and depots. Inhalation is the most common route of exposure, as gasoline

contains a variety of volatile compounds [1]. Crude oil is fractionally distilled to produce several petroleum fractions, including gasoline, kerosene, and diesel, which are constitutive elements. These crude oil fractions contain a variety of aliphatic, aromatic, saturated, and unsaturated hydrocarbons [2]. With a

variety of organic and inorganic components, gasoline is a particularly flammable liquid. Its components have been shown to contain several substances that are extremely poisonous or carcinogenic to humans. Many of the toxicological effects associated with exposure to gasoline can be attributed to specific components of gasoline, such as benzene, toluene, ethylene and xylene, which are also known as volatile organic compounds (VOCs) [3]. Petroleum products are often used in our daily lives; fuel attendants are required to dispense fuel from a reservoir [4]. Fuel attendants are exposed to gasoline by inhalation during refilling or through contaminated food at service stations [5].

Haematological parameters are useful for diagnosing many diseases and for assessing the extent of blood damage. They serve as a pathological reflector of the health of an individual that have been exposed to toxicants and other conditions [6]. Studies have also suggested a causal relationship between industrial exposure to benzene and the incidence of some types of leukaemia and aplastic anaemia [3]. The metabolism of aliphatic and aromatic hydrocarbons, which are the major constituents of crude petroleum and petroleum products, results in free radical species generation in various tissues. In red blood cells, free radicals are known to alter the red blood cell membrane due to oxidative stress. A large proportion of crude oil and other petroleum products are lipophilic in nature, and biological membranes may, therefore, be the target sites where their adverse effect occur [7]. Excessive benzene exposure has been shown to affect the bone marrow, resulting in a reduction in the amount of circulating blood cells, anaemia, and other health problems, such as aplastic anaemia, thrombocytopenia, and leucopaenia. Also, the effect is characterised by increased sensitivity to injury, bone marrow (BM) suppression, and infections due to a lack of leucopoiesis [8].

The coagulation pathway is used to monitor the body's ability in clot formation, which can also be affected by either acute or chronic exposure to petrol fumes, which may cause thrombocytopenia, cardiac arrest or heart attack caused by the presence of thrombosis in the coronary artery of the heart, convulsion, body weakness and loss of consciousness. It also negatively affects coagulation factors, fibrinolysis, and other coagulation tests [9]. Exposure to combustion-derived pollutants appears to trigger myocardial infarction (MI), and the majority of such events result from thrombus formation at the

site of an atheromatous plaque. Exposure to petroleum would increase in vivo platelet activation and enhance thrombus formation [10]. This study aims to confirm the effects of petroleum toxicity among occupational workers exposed to these volatile hydrocarbons and to offer solutions for protecting themselves from its toxicity.

Methodology

Study area

The study was conducted at Usmanu Danfodiyo Teaching Hospital in the Sokoto metropolis, Sokoto State, Nigeria. Sokoto State is located in the extreme Northwest of Nigeria. It shares borders with the Niger Republic to the North, Zamfara State to the East, Kebbi State to the southeast, and the Benin Republic to the West. The major indigenous tribes in the state are the Hausa and Fulani, as well as other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Igbos, and others. As of 2007, the state had a population of 3.6 million [11]. However, based on the population's annual growth rate of 2.5%, the projected population for Sokoto State in 2022 should be around 5.2 million [12].

Study design

The study is a cross-sectional study designed to include 40 fuel attendants exposed to gasoline and 40 unexposed controls. All subjects were enrolled in the study conservatively. Socio-demographic data of the patients were obtained using a semi-structured interview questionnaire, which included age, gender, and other socio-demographic factors. The study lasted from October to December.

Study population

A total of 80 participants were selected for the study. These consist of 40 fuel attendants, comprising males and females and 40 apparently healthy controls that met the inclusion criteria and consented to participate in the study.

Participants selection criteria

Inclusion criteria

- Subjects exposed to gasoline, age (18-50 years)
- Subjects who have worked for at least two years and above
- Subjects who had normal physical health
- Willingness of subjects to offer verbal or written informed consent to participate in the study.

Exclusion criteria

- Subjects who were above 45 years
- Subjects who worked for less than 2 years
- Subjects who didn't consent.

Informed consent

Written informed consent was sought from subjects using a standard protocol

Ethical approval: Ethical approval for the study was obtained from the Ministry of Health, Sokoto, ethical committee (appendix).

Sampling techniques and methods of data collection

A simple random sampling method was employed to select the subjects. The blood sample was collected from participants who worked in various fuelling stations across Sokoto town. All subjects who met the inclusion criteria were given a written informed consent for this study. A semi-structured interview questionnaire was administered to all consenting subjects for their socio-demographic information, such as years of exposure.

Specimen collection and processing

A total of 5 ml of blood was collected from the cubital fossa vein using a 5ml syringe and needle, and 2.5 ml was dispensed into an ethylene tetraacetic acid (EDTA) container. While 2.25 ml of the blood was taken into a sodium citrate container that contains 0.25ml in a ratio of 9:1 (blood to anticoagulant). It was centrifuged at 4000rpm for 15minutes to get the poor platelet plasma. The poor platelet plasma (PPP) was separated and placed into a cryovial. The full blood count was carried out in the Specialist Hospital, Sokoto, while the coagulation studies were carried out in Usmanu Danfodiyo Teaching Hospital, Sokoto, Nigeria.

Laboratory diagnosis

Assay of Full Blood Count Parameters Using Sysmex KX- 21N Haematological Analyzer Manufactured by Sysmex Corporation, Japan.

The EDTA anticoagulated blood samples were analysed using the Sysmex haematology analyser, using the Coulter Counter method developed by Wallace H. Coulter in 1940. The machine is a three-part auto analyser able to test 19 parameters per sample, including RBC count, Haemoglobin concentration, Haematocrit (HCT), Total White Blood

cells and 3-differentials, Platelet counts and other related parameters. Determination of Coagulation Parameters Using Reagent from Spectrum for diagnostic Industries, Ismaila, Egypt. PT (Recoplastin) Lot, SPTR C0108023. PTTK (Unicelin) Lot, SAPIT 0208023. The coagulation studies carried out included: partial thromboplastin measurements and activated partial thromboplastin measurements (PT, PTTK). The method for both tests [13].

Prothrombin time

The prothrombin time was carried out to estimate the intrinsic pathway, with the INR calculated using a formula

Principle

Plasma is added to a thromboplastin and calcium chloride reagent at 37 degrees Celsius, and the time taken for the blood to form is measured. The clotting time in seconds was converted to the international normalised ratio, usually by reference to a manufacturer-provided table or a formula.

$INR = (PT \text{ patient} / PT \text{ control}) ISI$

Procedure

1. The citrated blood was centrifuged using 3000rpm for 15 minutes
2. The citrated plasma was separated, and 100 μ L was placed in a glass tube and pre-warmed in the water bath for 2minutes
3. A micro pipette was used to pick 200ul of the reagent and added to the plasma
4. The timer was simultaneously set, and clot formation was observed for the period of 15 seconds [13].

Partial thromboplastin time with kaolin (PTTK)

The PTTK test is carried out to assess the intrinsic pathway of coagulation

Principle

Kaolin (surface activator) and platelet substitute (phospholipid) are incubated with the citrated plasma at 37 degrees Celsius for the desired time. Calcium chloride is added, and the time taken for the mixture to clot is measured.

Procedure

1. In a clean glass tube, 100 μ L of the PTTK reagent was placed and incubated at 37 degrees Celsius for 3 minutes

2. Then 100ul of the citrated plasma was added and mixed for 2 minutes
3. Prewarmed calcium was added in 100ul, and the time was set simultaneously
4. Clot formation was observed within the period of 21-38 seconds [13].

shows that there was no Significant difference at $p < 0.05$ in parameters, such as MCV, MCH, MCHC, WBC and MPV, while there was a significant difference in RBC, HGB and HCT

Table 1: Sociodemographic characteristics of the respondents (N=80)

Variable	Frequency	Percentage
Age		
18-26	53	66.3
27-32	12	15.0
33-38	7	8.8
39-44	4	5.0
45-50	4	5.0
Sex		
Male	40	50
Female	40	50
Marital status		
Single	71	88.8
Married	9	11.3
Tribe		
Hausa	59	73.8
Fulani	3	3.8
Yoruba	10	12.5
Igbo	3	3.8
Dakarkare	3	3.8
Zuru	2	2.5
Years of Exposure		
2-10	30	37.5
11-20	6	7.5
21-30	3	3.8
31-40	1	1.3

Key: N; number

Statistical analysis

Data were recorded in an Excel spreadsheet, and statistical analysis was performed using SPSS version 27 on a computer. Results were expressed as mean (standard deviation), and Student's t-test and Pearson's correlation were used for testing. A p-value at $p < 0.05$ will be considered statistically significant

Results

Table 1 shows the socio-demographic distribution of the study population. Of the 73.8% who were Hausa young adults aged 18-26, up to 66.3% were males who participated in the study, compared to females. Up to 88.8% of participants in the study were single, and the years of exposure ranged mostly from 2 to 10 years. Table 4.2. Shows the mean \pm SD of some haematological parameters and also their t and p values of both the exposed and unexposed groups. The haematological parameters for the unexposed group showed normal values for RBC, HGB, HCT, MCH, MCHC, PLT, MPV, and WBC, but a low MCV that was not significant. Comparison between the groups

Table 4.2: Comparison of some haematological parameters for the exposed and unexposed groups and their mean \pm sd.

Parameters	Number	Exposed Mean \pm SD	Unexposed Mean \pm SD	t-value	p-value
RBC	40	5.77 \pm 0.78	5.01 \pm 0.64	4.77	0.0001*
Hb	40	13.27 \pm 1.48	11.81 \pm 1.56	4.297	0.0001*
HCT	40	41.89 \pm 5.19	38.02 \pm 4.13	3.695	0.0001*
MCV	40	72.74 \pm 4.25	74.71 \pm 6.04	-1.690	0.095
MCH	40	23.13 \pm 2.21	23.17 \pm 2.83	-0.071	0.944
MCHC	40	31.83 \pm 2.48	31.07 \pm 2.70	1.299	0.198
PLT	40	305.58 \pm 102.25	312.25 \pm 94	-0.304	0.762
WBC	40	6.11 \pm 2.99	5.19 \pm 1.72	1.689	0.095
MPV	40	10.87 \pm 1.06	11.32 \pm 1.35	-1.658	0.101

Legend: Values are mean \pm standard deviation, N = number of subjects; SD = Standard deviation; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular Haemoglobin concentration; PLT = platelet; WBC = white blood cell count; MPV = mean platelet volume. * indicate $p < 0.05$ is considered statistically significant.

Table 4.3. Shows the mean \pm SD of some coagulation parameters and also their t and p value for both the exposed and unexposed groups. The mean values for the coagulation parameters in the exposed group showed normal values in PT, PTTK and INR were within normal range for the unexposed group, while the PTTK values for the exposed group were statistically

abnormal, but the PT and INR were within normal range in the exposed group. The PT and INR of the exposed group was significantly lower compared to the unexposed group while the PTTK of the exposed group was significantly increased in the exposed group compared to the unexposed group ($p < 0.05$).

Table 4.3: Comparison of some coagulation parameters for the exposed and unexposed group and their mean±SD.

Parameters	Number	Exposed Mean±SD	Unexposed Mean±SD	t-value	p-value
PT	40	13.03±0.28	14.25±1.32	-5.763	0.0001*
PTTK	40	41.13±8.37	30.28±5.61	6.811	0.0001*
INR	40	1.002±0.02	1.096±0.09	-5.859	0.0001*

Legend: Values are mean ± standard deviation, N = number of subjects; SD = Standard deviation; PT = prothrombin time; PTTK = partial thromboplastin time with kaolin; INR= international normalised ratio. * indicate p<0.05 is considered statistically significant.

Table 4.4. Shows correlation between the haematological and coagulation parameters among the exposed and the unexposed groups. It shows that there was a negative correlation between the PT, RBC and PT, HCT, which was statistically significant. A negative statistically significant correlation was seen between INR, RBC and INR, HCT

Table 4: Correlation between haematological and coagulation parameters between exposed and unexposed groups

Parameters	PT	PTTK	INR
RBC r	-0.295*	0.168	0.301*
P	0.008	0.137	0.007
Hb r	-0.119	-0.117	-0.120
P	0.292	0.302	0.287
HCT r	-0.310*	0.128	-0.315*
P	0.005	0.259	0.004
MCV r	0.092	-0.033	0.095
P	0.415	0.771	0.403
MCH r	0.092	0.100	-0.060
P	0.415	0.380	0.596
MCHC r	0.064	0.143	-0.184
P	0.573	0.207	0.103
PLT r	0.012	-0.117	0.012
P	0.914	0.301	0.918
WBC r	-0.064	0.148	-0.062
P	0.594	0.191	0.587
MPV r	0.172	-0.133	-0.062
P	0.128	0.238	0.587

Legend: r; regression, (positive values means both parameters increases and decreases at the same time while negative value means one increases and the other decreases or vice-versa) RBC; red blood cell, Hb, haemoglobin, HCT; haematocrit; MCV; mean cell volume, MCH; mean corpuscular haemoglobin, MCHC, mean corpuscular Haemoglobin concentration, PLT; platelet, WBC; white blood cell count, MPV; mean platelet volume, PT; prothrombin time, PTTK; partial thromboplastin time with kaolin, INR; international normalized ratio. p<0.05 is considered statistically significant.

Discussion

This research was to assess the effect of gasoline exposure on haematological and coagulation parameters among fuel attendants in Sokoto town of Nigeria. The total of 80 participants were recruited for the study, which comprised of 20 females and 20 males which made up of 40 exposed group and 40 unexposed which comprised of 20 females and 20 males.

In this Present study, the RBC, Hb and HCT in this study showed a significant increase in the exposed compared to the unexposed. This study agreed with a study conducted by Ahamdi [14], which was conducted in Iran on “The Association of

environmental exposure with hematological and oxidative stress alteration in gasoline station attendants” which showed a significant increase in the RBC, Hb and HCT of the male subjects with a p value of (<0.001). A study by Alsas et al. [1], on “Evaluation of some biological parameters of gasoline station attendants in Damascus, Syria” which also showed a statistically significant increase in RBC, Hb, and HCT, with a p value of (p<0.05). Also, previous studies by Awodele et al. [4], on “Evaluation of haematological, hepatic and renal functions of petroleum tanker drivers in Lagos, Nigeria”, with a p value of (<0.05). The increase in RBC, Hb and HCT

were within the normal range and this indicates that the gasoline did not cause a negative effect in the RBC, Hb and HCT of the exposed. This could be as a result of shorter working hours as they work for 5 hours daily. It could also be because 37.5% of the exposed had shorter years of exposure which ranged from 2-10 years. This increase might also be because most of the exposed were males who tend to have higher RBC, Hb and HCT values. Contrarily, a study by Teklu *et al.* [5], on “Effect of Gasoline Exposure on Haematological Parameters of Gas Station Workers in Mekelle City, Tigray Region, Northern Ethiopia” with a statistically significant p value of (<0.05). The decrease in this study could be because the exposed group in the study had longer working hours of up to 12 hours daily which might have caused the decrease in the RBC, Hb and HCT levels. It could also be due to difference in geographic regions as this study was carried out in Ethiopia who might be using a different gasoline product compared to my study done in Nigeria. Previous study by Okoro *et al.* [15], on “Effect of Petroleum Products Inhalation on Some Haematological Indices of Fuel Attendants in Calabar Metropolis, Nigeria” which showed a decrease in RBC, HCT and Hb with a statistically significant p value of (<0.001), the decrease in RBC, Hb and HCT in the exposed subjects in this study, could be as a result of the geographic region as the study was carried out in Calabar metropolis where they tend to be more exposed aside from inhalation as there are many cases of petrol pipeline leakages which may have led to the decrease in RBC, Hb and HCT. Also, it may be due to the match sex distribution used in the study which had same number of male and female subjects, as females are known to have lower RBC, Hb and HCT values. This may have caused the decrease in RBC, Hb, and HCT in the study.

The MCH and MCHC did not show a statistically significant difference in the exposed subject compared to the control. This study agreed with a study by Abou- ElWafa *et al.* [16], on “Some Biochemical and Haematological Parameters among Petrol Station Attendants: A Comparative Study” which showed similar result in the exposed and control group but was not statistically significant. This could be because there was no sufficient exposure to the gasoline that could cause alterations in the MCH and MCHC. Contrarily, a study by Jabbar and Ali [17], on “Impact of Petroleum Exposure on Some Haematological Indices, Interleukin-6, and Inflammatory Markers of Workers at Petroleum

Stations in Basra City” showed a statistically significant decrease in MCH and MCHC compared to the control with a p value of (<0.05). The decrease in the MCH and MCHC in this study could be due to the higher working hours of the exposed to gasoline and as a result of the difference in geographic region as Iraq is popularly known for the production of gasoline which may have led to a higher exposure resulting to decrease in the MCH and MCHC values of the study compared to this study. The MCV in this study showed a decreased in the exposed compared to the control group which was not significant. This study agreed to a study by Anyiam *et al.* [18], on “Effects of gasoline on haematological parameters of gasoline station workers in Onitsha, Anambra State, Nigeria” which showed a decrease in MCV in the exposed compared to the control group which was not statistically significant ($p>0.05$). This may be due to the lower exposure time which may not have caused any negative changes in the MCV of the exposed. In contrast, a study by Teklu *et al.* [5], in “Impact of Petroleum Exposure on Some Haematological Indices, Interleukin-6, and Inflammatory Markers of Workers at Petroleum Stations in Basra City” Showed a significant increase in the MCV of the exposed compared to the control group. Also, previous study demonstrated that the toxic ingredient in petroleum, including benzene and lead are activated in the bone marrow, where the substances exert cytotoxic effects that could be mediated to change DNA function. A defect in DNA synthesis that interferes with cellular proliferation and maturation can lead to destruction and change in the shape of erythrocytes [5]. The increase in MCV in this study can be due to macrocytosis induced by benzene and benzene is an ingredient of gasoline due to longer exposure to gasoline.

The PLT and the MPV in this study were decreased in the exposed group compared to the control but was not statistically significant. This agreed to a study by Teklu *et al.* [5], on “Effect of Gasoline Exposure on Haematological Parameters of Gas Station Workers in Mekelle City, Tigray Region, Northern Ethiopia” which showed a decrease in the PLT of the exposed compared to the control but was not statistically significant at p value of (<0.05). The decrease in PLT and MPV may be due to the toxic effect of Benzene, as Benzene is one of the main constituents of gasoline, it is a well-known systemic toxicant in humans and a cause of aplastic anaemia. It is haematotoxic and depresses the bone marrow, leading to pancytopenia, depression of erythrocytes (red blood cells),

leucocytes (white blood cells) and thrombocytes (platelets). These studies demonstrate that benzene is indeed a haematotoxicant which may have caused the decrease in PLT and MPV. However, the exposure to gasoline was not sufficient to cause a significant decrease in this study.

The WBC in this study showed an increase in the exposed group compared to the control which was not statistically significant. This agreed to a study by Ajugwo *et al.* [19], in “Reduced Haematological Indices in Auto-Mechanics and Fuel Attendants in Elele Nigeria” which showed an increase in the WBC of the exposed when compared to the control group which was not statistically significant (>0.05). The increase in WBC may be due to immune response elicited by toxic effect of benzene on WBC. However, due to the shorter time of exposure in the exposed there was no significant increase. In contrast to this study, a study on “Assessment of Haematological Parameters Among Petrol Station Attendants and Auto Mechanics in Port Harcourt Metropolis, Rivers State” showed a significant decrease in the WBC of the exposed group compared to the control. The decrease in WBC in this study could be due to the depression of the bone marrow caused by the effect of benzene, leading to pancytopenia and depression of leucocytes (white blood cells) as the geographic region is a location for mining of crude oil which may have resulted to the significant decrease in WBC.

The PTTK of the exposed showed significant increase compared to the unexposed group agreed with a study by Okeke *et al.* [9], on “Impact of occupational exposure to petroleum products on coagulation and white blood cell indices of Petrol station attendants in Nnewi metropolis, Nigeria” which showed a statistically significant increase in the PTTK of the exposed compared to the control group at $p(<0.05)$. The increase in PTTK indicates that gasoline has a toxic effect on the intrinsic pathway due the toxic effect of Benzene which is one of the volatile organic compounds in petrol and has been linked to thrombocytopenia due to its damage to the DNA of pluripotential stem cells. However, the mechanism behind this is not clearly understood but may be linked to the effect of the toxic components of petroleum product fumes to the haemostatic mechanism of the body.

The PT and INR in this study showed a statistically significant decrease in the PT and INR in the exposed group compared to the unexposed group. However, it was within the normal range. It agreed with a study by

Okeke *et al.* [9], on “Impact of occupational exposure to petroleum products on coagulation and white blood cell indices of Petrol station attendants in Nnewi metropolis, Nigeria” at p value (<0.05). The decrease in the PT and INR could as result of the toxic effect of the constituent of gasoline on the coagulation proteins in the extrinsic pathway of coagulation but it was not sufficient to cause a decrease below the normal range. The Correlation between the haematological and coagulation parameters among the exposed and the unexposed groups shows that there was a negative correlation between the PT and RBC, HCT. Which was statistically significant but no statistically significant correlation between PT and the rest of the haematological parameters. No statistically significant correlation was seen in PTTK and haematological parameters. A negative statistically significant correlation was seen between INR and RBC, HCT. at $p<0.05$. Due to scarcity of work done on correlation between both parameters, the study could not be compared or contrasted by other research on both parameters.

Conclusion

From these findings, it indicated that gasoline had no effect on the haematological parameters of the fuel attendants but had an effect on their coagulation parameters. Hence, we recommend that the petrol station owners should minimize the exposure of their employees to gasoline by providing protective equipment.

Recommendation

1. Further studies should be done on sex matched subjects as these parameters has different ranges based on gender.
2. More research should be done on coagulation parameters among fuel station workers as there are very few papers available on this study.
3. Further studies should be done to determine the mechanism of action by which gasoline affects coagulation.

Acknowledgement: I would like to sincerely appreciate all the authors who contributed financially to the payment of publication for this manuscript. I would also especially like to thank all the authors for their constant support, direction, and encouragement.

Conflict of Interest: The authors declare that they have no competing interest

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