DOI: https://doi.org/10.53350/pjmhs2023178216

ORIGINAL ARTICLE

Investigation of Hereditary Thrombophilia in Women with Recurrent Fetal Loss in LUMHS Hyderabad; A Descriptive Cross-Sectional Study

KAYNAT ILYAS[1], SUGHAND KHASKHELI[2], BENAZEER BHATTI[3], RIYASAT AHMED MEMON[4], RAMEEZ IQBAL MEMON[5], SAMINA[6], MARVI[7]

[1-3] *Postgraduate Student, Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro*
[4] *Assistant Professor, Department of Pathology, Bilawal Medical College for Boys, LUMHS, Jamshoro*
[5] *Lecturer, Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro*
[6] *Professor, Department of Gynecology & Obstetrics, Jinnah Medical College, Peshawar*
[7] *Assistant Professor, Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Baluchistan, Quetta,*
Correspondence to: Kaynat Ilyas, Email: kaynatkhanzada@gmail.com

ABSTRACT

Objectives: To evaluate the role of Antithrombin, Protein C, Protein S and Factor V Leiden in recurrent pregnancy loss and to compare the cause of hereditary thrombophilia among women with recurrent pregnancy loss.

Methods: Sixty patients with two or more pregnancy loss having ages 19-40 years were included in the descriptive cross-sectional study was performed at Diagnostic and Research Laboratory LUMHS Jamshoro/Hyderabad and Department of Pathology LUMHS, Jamshoro from January 2020 to December 2020. While patients with liver disease, uterine Fibroids, Cardiac disease, congenital abnormalities associated pregnancy loss were excluded.

Total 10 ml blood was taken and distributed in EDTA tube and in sodium citrate. CBC was performed on automated CBC analyzer Sysmex CA 1000. PT, APTT performed on Sysmex CA 600. Antithrombin screening done by INNOVANCE Antithrombin Kit. Protein C activity measured by Berichrome Protein C Kit. Protein S screened by Siemens Protein S Ac kit. Factor V Leiden was done by using Pro C global kit with factor V deficient plasma. Quality control and standard curves were performed as per kits instructions.

Results: In our study of sixty women with recurrent fetal loss, mean ages were 29.3± 4.9. The mean number of fetal loss among patients was (3.1± 1.5) while parity was (1.6 ± 1.2). Among these patients mean platelets were (302.6 ± 74.9), PT was (11.9 ± 0.14), APTT was (27.1 ±1.3). Protein C was found in 12 (20%), while 19 (31%) patients had Factor V Leiden. Whereas, Protein S was found in 3 (5%) patients. Antithrombin was not detected in any patients.

Conclusion: Our research based on the results concludes that Factor V Leiden had a significant association to the women with recurrent fetal loss and it could be a cause of recurrent pregnancy loss.

Keywords: Hereditary thrombophilia, Recurrent fetal loss, Factor V leiden.

INTRODUCTION

American Society of Reproduction Medicine (ASRM) defines, the recurrent miscarriage (RM) is the pregnancy failure which occurs at least two times[1]. RM may be categorized as either early (miscarriages which happen during 20 weeks of pregnancy) or late (miscarriages which occur after first 20 weeks of pregnancy)[2]. RM is a usual health issue, with at least three miscarriage influencing 1-2% and at least two miscarriages occurs into 5% of women at the time of reproduction[3].

Pregnancy is a hypercoagulable condition if it is influenced by thrombophilia, the hypercoagulable condition gets severe and may hinder the blood circulation via the maternal veins, resulting in deep vein thrombosis (DVT), and formation of clumps in the placental circulation, which results in reduced fetal growth and/or it can lead to fetal loss[2]. Low quantity of natural anticoagulants enhances the thromboembolism risk throughout the pregnancy[4].

Thrombophilia can be defined as a set of disorders which distinguishes the reduction in the anticoagulant mechanism, resulting predisposition to enhancement in thromboembolism. Thrombophilia could be acquired or inherited/genetics[5]. “Hereditary” or “inherited” thrombophilia is the most common terminology applicable to such states in which a hereditary change influences the quantity/role of a protein in the thrombotic pathway. Antithrombin (AT), Protein C (PC) and Protein S (PS) have the loss of function mutations while the Factor V Leiden (FVL) and the prothrombin gene 20210 A/G have gain of function mutations[6]. Heterozygote or homozygote change in FVL, PS deficiency, PC deficiency, or AT- III were explained as the reasons for an increased rate of fetal loss[7].

Thrombophilia mechanism depends on three factors. One is more chances of thrombophilia in recurrent fetal loss (RFL), Second is the high rate of miscarriage due to thrombophilia and the presence of clot formation in placental circulation[8].

Received on 15-03-2023
Accepted on 22-07-2023

Pregnancy is the physiological condition that changes the hemostatic state into hypercoagulability. It causes changes in blood, vessels and coagulation with high quantity of factor VII, VIII, X, Von Will brand Factor (VWF), fibrinogen, thrombin generation markers F1, F2 and thrombin AT complex. There is reduced concentration of PC, PS, and APC and fibrinolytic activity. During pregnancy, these changes have a protective role but it can lead to complications particularly more chances in women having acquired or hereditary thrombophilia[9,10].

Equilibrium between pro coagulant and anticoagulant system is needed for sufficient placental blood flow and pregnancy. Reduced placental blood flow leads to thrombosis, reduced oxygen supply and invasion of trophoblast and it causes fetal growth restriction, placental abruption and pregnancy loss[11,12]. For women health fetal loss is a big issue. Studies showing 1/5th women having one pregnancy loss and 1/20 having two and more abortions. One (1) % of women have minimum three recurrent miscarriages[13,14].

ASRM suggests at least two pregnancy loss, while European Society of Reproduction and Embryology suggests at least three pregnancy loss. Recent studies showed two or more pregnancy loss as habitual or recurrent irrespective of it is succeeding or not[15,16]. In 2005, improved nomenclature says Recurrent pregnancy loss (RPL) is consider as early loss which occurs prior to 12 weeks’ pregnancy and consider as late losses which happens after 12 weeks. RPL also described as loss of pregnancy at or prior to 20 weeks or loss of pregnancy after 20 weeks, with 8-20% take place prior to 20 weeks’ gestation while in this 80% within 12 weeks of gestation[2]. Miscarriages has a psychological impact on women health, among which 14 to 19% are associated with one miscarriage while two or more pregnancy loss occur in 1-5%. In greater than fifty percent reasons left undetermined. Many types of genetic thrombophilia have related to fetal loss but this connection is still unclear[16].

Fetal loss occurs in any trimester has a deleterious effect on women going through it both in terms of psychological and social. Several women having pregnancy loss without knowing the fact

that they are having pregnancy therefore actual ratio of pregnancy loss is more than that is published. 40-50% cases showing association of thrombophilia with recurrent fetal loss[2]. Although genetic thrombophilia showing association with recurrent fetal loss, but this relationship among genetic thrombophilia and recurrent fetal loss has not been clearly determined. Mostly research has been published on Caucasian papulations on relationship of thrombophilia and RPL[17]. Clinicians has a hope that anticoagulant treatment will be beneficial in management of the patients having recurrent fetal loss due to inherited thrombophilia and leads them to include inherited thrombophilia screening as a routine investigation in antenatal checkup in patients with fetal loss[18].

Studies showing different results for relation of thrombophilia with fetal loss due to the change in chosen criteria for patients and also because different frequency of inherited thrombophilia in selected community[4]. In our region frequency and cause of hereditary thrombophilia in recurrent fetal loss is not described and need to be established. The aim of our study is screening of hereditary thrombophilia among females with recurrent fetal loss.

MATERIAL AND METHOD

This descriptive cross-sectional study was performed at Diagnostic and Research Laboratory LUMHS Jamshoro/Hyderabad and Department of Pathology LUMHS, Jamshoro from January 2020 to December 2020. A total sixty (60) patients having two and more unexplained pregnancy loss were selected for study. The identification of pregnancy loss was taken on the basis of history, medical reports, U/S reports and the laboratory investigation provided by the patient.

Inclusion Criteria: Patients having two and more fetal loss included in the study. Patients having ages 17-45 were also included in the study.

Exclusion Criteria: Women who have history of liver disease, uterine fibroid, polycystic ovaries (PCO) and cardiac disease were excluded from study. Patients with congenital abnormalities associated pregnancy loss were also excluded from study.

Data Collection Procedure: Total 10 ml of blood specimen was taken from individual patient and diffused into EDTA (Ethylene diamine tetra acetic acid) vacutainer for complete blood count (CBC) and in 3.2% sodium citrate tube in a ratio of 9:1 for coagulation assays with adequate mingling of six (6) times avoiding vigorous shaking. Complete blood count (CBC), Prothrombin time (PT) and Activated partial thromboplastin time (APTT) of all patients were performed on the same day. All samples processed on same day during 2 hour of sample collection through twice centrifuged at 2000g for 15 minutes to obtained platelet poor plasma and placed in aliquots at -35℃ for factor analysis.

Laboratory Analysis: CBC was performed on analyzer Sysmex XN-1000. PT, ATT analyzed on coagulation analyzer (Sysmex CA-600) on fresh sample of platelet free plasma. PC was measured by using Berichrome PC kit Siemens Healthcare Diagnostic (Sysmex CA-1500). FVL screening was performed through Pro C Global kit in conjugation with Factor V deficient plasma on Sysmex CA-1500 analyzer.

Antithrombin activity was measured by using INNOVANCE Antithrombin kit on automated analyzer Sysmex CA-2500. Protein S was screened by using Siemens Protein S Ac kit on Sysmex CA-2500. Commercial control plasma N, P and standard human plasma were used for quality control and standard curve preparations.

Statistics: Data analysis was carried out by using SPSS version 22. The frequency and percentages were calculated for the factors (AT, PC, PS and FVL). The mean ± standard deviation (SD) was calculated for age, parity, RPL, platelets, PT, APTT and for the factors.

RESULTS

A total sixty women with RFL were included in the study. Thirty patients (50%) presented with two fetal losses, 12 (20%) with three fetal losses and 18 (30%) with more than 3 fetal losses. Among these women 26 (43.3%) had 1st trimester losses, 14 (23.3%) had second trimester losses, 10 (16.6%) had combined losses.

All patients were aged between 19-40 years (mean 29.3± 4.9). The mean number of fetal loss among patients was (3.1± 1.5) while parity was (1.6 ± 1.2). Among these patients mean platelets were (302.6 ± 74.9), PT was (11.9 ± 0.14), APTT was (27.1 ±1.3). Table 1

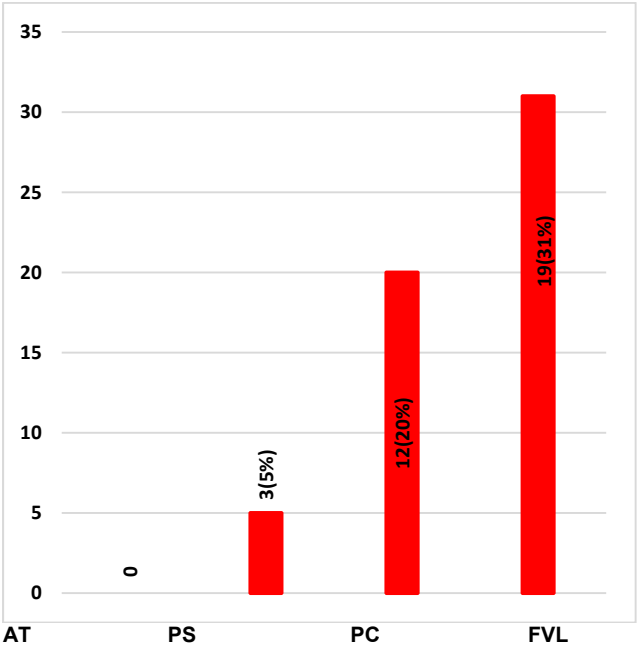
In these women with recurrent fetal losses 51.6% presented with hereditary thrombophilia. PC deficiency was presented in 12 (20%) cases, PS was identified in 3 (5%) while AT was not detected in any patient. FVL was identified in 19 (31%) cases (Table 2). Among which 10 (16.6%) patients showed combined deficiencies, nine had PC and FVL deficiency and one case having simultaneous PC and PS deficiency.

Table 1. Demographic data and coagulation results

Parameter	Patients (n=60) (mean)
Age	29.3± 4.9
RPL	3.1± 1.5
Parity	2.2 ± 2.0
Platelets	302.6 ± 74.9
PT	11.9 ± 0.14
APTT	27.1 ±1.3

Table 2. Frequency of Hereditary thrombophilia in recurrent fetal loss

Parameter	Frequency	Mean
PC	20%	71.2±14.0
PS	5%	72.5±10.1
AT	0%	97.6±11.1
FVL	31%	0.77±0.11



DISCUSSION

In this study, we compared the cause of thrombophilia among women with RFL and relative risk of hereditary thrombophilia because of AT, PC, PS and FVL. Hereditary thrombophilia is a coagulation defect. Most commonly occurring hereditary factor deficiency is FVL which is present in 40% among European papulation. While AT, PC, PS occur in 5 to 10% of patients with thromboembolism[19]. Pregnancy loss is a major issue. Due to pregnancy itself is an acquired hypercoagulable condition, hereditary factors that leads to thrombosis increased the chances

of complications. Many studies enrolled to see the association between hereditary thrombophilia and worse end pregnancy effects that showed different results[20].

Thromboprophylaxis is considered as a therapy for patients with recurrent fetal loss. The widespread use of anticoagulant prophylaxis treatment for women with thrombophilia associated worse end pregnancy results has led to overcome over the lack of literature in favors of using this therapy. There is not much supportive data due to difference of study papulation, complications and trimester at which this therapy is given in these studies[12]. The ACCP (American college of chest physician) does not favor the utilization of LMWH in women have no family history of VTE or women having family history of VTE in association with any other hereditary thrombophilia and does not recommend prophylactic use in women of hereditary thrombophilia in absence of adverse pregnancy outcome in past (21). While RCOG guidelines supported the usage of LMWH prophylaxis in women having AT, PC, PS deficiency, homozygous for FVL and prothrombin G20210A without the presence of family history[22].

In our study, hereditary thrombophilia was found in 51% of women with recurrent fetal loss. The percentages are different among studies. These controversies in results may be due to difference in selection of patients, ethnic background, inclusion criteria, study design, population and geographical areas. Nahas et al found thrombophilia in 53.3%[7]. while other two studies showing frequency between 49%, 65% and 66%[23-25].

In our study group frequency of Protein C was 20% while other studies showed variable prevalence which are from 6%, 7.7%, 22.5% respectively[5,7,26]. Protein S was identified in 5% of patients finding similar to other studies[1,4,26]. Antithrombin was not detected in any patient while previous studies favor our findings[1,17], whereas low prevalence was shown in other studies from 0.2%, 0.6% and 1.9%[4,7,26].

FVL was widely studied as a reason of recurrent fetal loss. The prevalence of FVL in recurrent fetal loss from different regions of world showed 3% to 40%[11,25,27,28].

In a review article of fourteen[14] researches, Nasibeh et al evaluated that most of them showed the connection among FVL and fetal loss[29].

Another review article which includes 62 studies also indicated a significant relationship of FVL and risk of RPL in Asians, European and African population[30].

Assaad et al concludes that FVL mutation could be the cause of recurrent fetal loss[31]. Another recent study shows an important connection between FVL and RFL especially in 1st and 2nd month of pregnancy[32]. Although some studies showed no relationship between FVL mutation and RPL[33,34].

In our research, FVL was identified in 31% of women with recurrent fetal loss. Very few studies have been conducted in Pakistan. In comparison to our data a study from Pakistan showed 27% of FVL in women with RPL results favoring our findings[26]. Another study from northern Pakistan, Nadir et al found 12% FVL[4].

Most of the studies favors that FVL has significant association with recurrent fetal loss. Our data gave further understanding about testing of hereditary thrombophilia in RFL and it will be helpful for further investigation regarding role of anticoagulants in recurrent fetal loss. It could be beneficial in timely treatment of patients through proper thromboprophylaxis and thereby improves maternal and perinatal outcome.

CONCLUSION

Our research based on results, concludes that FVL had a significant association to the women with recurrent fetal loss and it could be a cause of recurrent pregnancy loss.

Limitation of Study: Factor V mutation should be done on PCR. Further studies with large population should be conducted to screen patients with recurrent fetal loss and awareness should be created among health professionals regarding role of hereditary thrombophilia and to screen the patients with recurrent fetal loss. Early diagnosis will help in proper management of patients

REFERENCES

1. Kumar N, Ahluwalia J, Das R, Rohilla M, Bose S, Kishan H, et al. Inherited thrombophilia profile in patients with recurrent miscarriages: Experience from a tertiary care center in north India. Obstetrics & gynecology science. 2015;58(6):514.
2. Abu-Heija A. Thrombophilia and Recurrent Pregnancy Loss: Is heparin still the drug of choice? Sultan Qaboos Univ Med J. 2014;14(1):e26-36.
3. Parand A, Zolghadri J, Nezam M, Afrasiabi A, Haghpanah S, Karimi M. Inherited thrombophilia and recurrent pregnancy loss. Iranian Red Crescent medical journal. 2013;15(12).
4. Ali N, Bhatti FA, Khan SA. Frequency of hereditary thrombophilia in women with recurrent pregnancy loss in N orthern P akistan. Journal of Obstetrics and Gynaecology Research. 2014;40(6):1561-6.
5. Hossain N, Shamsi T, Soomro N. Frequency of thrombophilia in patients with adverse pregnancy outcome. JOURNAL-PAKISTAN MEDICAL ASSOCIATION. 2005;55(6):245.
6. Stevens SM, Woller SC, Bauer KA, Kasthuri R, Cushman M, Streiff M, et al. Guidance for the evaluation and treatment of hereditary and acquired thrombophilia. Journal of thrombosis and thrombolysis. 2016;41(1):154-64.
7. Nahas R, Saliba W, Elias A, Elias M. The prevalence of thrombophilia in women with recurrent fetal loss and outcome of anticoagulation therapy for the prevention of miscarriages. Clinical and Applied Thrombosis/Hemostasis. 2018;24(1):122-8.
8. Hansda J, Roychowdhury J. Study of thrombophilia in recurrent pregnancy loss. The Journal of Obstetrics and Gynecology of India. 2012;62(5):536-40.
9. Coriu L, Ungureanu R, Talmaci R, Uscatescu V, Cirstoiu M, Coriu D, et al. Hereditary Thrombophilia and thrombotic events in pregnancy: single-center experience. Journal of medicine and life. 2014;7(4):567.
10. Simcox LE, Ormesher L, Tower C, Greer IA. Thrombophilia and pregnancy complications. International journal of molecular sciences. 2015;16(12):28418-28.
11. Vossen C, Preston F, Conard J, Fontcuberta J, Makris M, Van Der Meer F, et al. Hereditary thrombophilia and fetal loss: a prospective follow-up study. Journal of Thrombosis and Haemostasis. 2004;2(4):592-6.
12. Ormesher L, Simcox L, Tower C, Greer IA. Management of inherited thrombophilia in pregnancy. Women's Health. 2016;12(4):433-41.
13. Bogdanova N, Markoff A. Hereditary thrombophilic risk factors for recurrent pregnancy loss. Journal of community genetics. 2010;1(2):47-53.
14. Lund M, Nielsen H, Hviid T, Steffensen R, Nyboe Andersen A, Christiansen O. Hereditary thrombophilia and recurrent pregnancy loss: a retrospective cohort study of pregnancy outcome and obstetric complications. Human reproduction. 2010;25(12):2978-84.
15. Liatsikos SA, Tsikouras P, Manav B, Csorba R, von Tempelhoff GF, Galazios G. Inherited thrombophilia and reproductive disorders. Journal of the Turkish German Gynecological Association. 2016;17(1):45.
16. de Jong PG, Goddijn M, Middeldorp S, editors. Testing for inherited thrombophilia in recurrent miscarriage. Seminars in reproductive medicine; 2011: © Thieme Medical Publishers.
17. Cardona H, Castañeda SA, Cardona Maya W, Alvarez L, Gómez J, Gómez J, et al. Lack of association between recurrent pregnancy loss and inherited thrombophilia in a group of Colombian patients. Thrombosis. 2012;2012.
18. Mitić G, Novakov-Mikić A, Považan L, Mitreski A, Kopitović V, Vejnović T. Thromboprophylaxis implementation during pregnancy in women with recurrent foetal losses and thrombophilia. Medicinski pregled. 2011;64(9-10):471-5.
19. Martinelli I, Mannucci PM, De Stefano V, Taioli E, Rossi V, Crosti F, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. Blood, The Journal of the American Society of Hematology. 1998;92(7):2353-8.
20. Kocher O, Cirovic C, Malynn E, Rowland CM, Bare LA, Young BA, et al. Obstetric complications in patients with hereditary thrombophilia identified using the LCx microparticle enzyme immunoassay: a controlled study of 5,000 patients. American journal of clinical pathology. 2007;127(1):68-75.
21. Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos A-M, Vandvik PO. VTE, thrombophilia, antithrombotic therapy, and pregnancy: antithrombotic therapy and prevention of thrombosis: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2):e691S-e736S.

22. Obstetricians RCo, Gynaecologists. Reducing the risk of venous thromboembolism during pregnancy and the puerperium. Green Top Guideline. 2015(37a).
23. Brenner B. Inherited thrombophilia and pregnancy loss. Thrombosis and haemostasis. 1999;82(08):634-40.
24. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. New England Journal of Medicine. 1999;340(1):9-13.
25. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertility and sterility. 2002;77(2):342-7.
26. Hossain N, Shamsi T, Khan N, Naz A. Thrombophilia investigation in Pakistani women with recurrent pregnancy loss. Journal of Obstetrics and Gynaecology Research. 2013;39(1):121-5.
27. Preston F, Rosendaal F, Walker I, Briet E, Berntorp E, Conard Ja, et al. Increased fetal loss in women with heritable thrombophilia. The Lancet. 1996;348(9032):913-6.
28. Pickering W, Marriott K, Regan L. G20210A prothrombin gene mutation: prevalence in a recurrent miscarriage population. Clinical and Applied Thrombosis/Hemostasis. 2001;7(1):25-8.
29. Roozbeh N, Banihashemi F, Mehraban M, Abdi F. Potential role of Factor V Leiden mutation in adverse pregnancy outcomes: an updated systematic review. Biomed Res Ther. 2017.
30. Eslami MM, Soufizomorrod M, Abroun S, Razi B. Factor V Leiden 1691G> A mutation and the risk of recurrent pregnancy loss (RPL): systematic review and meta-analysis. Thrombosis Journal. 2020;18(1):1-16.
31. Babker A, Gameel F, Elzaki S. Heterozygosity of maternal factor V G1691A (Leiden) and relationship with times of pregnancy loss among unexplained recurrent pregnancy loss women. Hematol Transfus Int J. 2018;6(5):208-10.
32. Nassour-Mokhtari I, Loukidi B, Moussouni A, Bettioui R, Benhabib R, Merzouk H, et al. Inherited thrombophilia and recurrent pregnancy loss: a single-center case-control study in North-Western Algeria. Egyptian Journal of Medical Human Genetics. 2020;21(1):1-7.
33. Pasquier E, Bohec C, Mottier D, Jaffuel S, Mercier B, Férec C, et al. Inherited thrombophilias and unexplained pregnancy loss: an incident case-control study. Journal of Thrombosis and Haemostasis. 2009;7(2):306-11.
34. Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, et al. Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: data from the southeast of Turkey. Annals of hematology. 2007;86(10):727-31.

This article may be cited as: Ilyas K, Khaskheli S, Bhatti B, Memon RA, Memon RI, Samina, Marvi; Investigation of Hereditary Thrombophilia in Women with Recurrent Fetal Loss in LUMHS Hyderabad; A Descriptive Cross-Sectional Study**.** Pak J Med Health Sci, 2023;17(8): 216-219.