**The University of Zambia**
**School of Medicine**
**Department of Pathology & Microbiology**

# Circulating Microparticles: A Risk Factor For Recurrent Abortion In Women At The University Teaching Hospital, Lusaka, Zambia.

**By**

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**A Research Dissertation Submitted To The University Of Zambia In Partial Fulfilment Of The Requirements For The Degree Of Master Of Science In Pathology (Haematology)**

**The University of Zambia**
**Lusaka**

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## Declaration

I, **Chibuye Sylvia**this 22nd day of August 2016, declare that this dissertation represents my own work. This work has not been done in Zambia before and neither has it been published for any qualification at the University of Zambia or any other University. Various sources to which I am indebted are clearly indicated in the text and in the references.

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## Certification of Approval

**Dissertation Title: Circulating Microparticles: A Risk Factor For Recurrent Abortion In Women At The University Teaching Hospital, Lusaka, Zambia.**

This dissertation for Chibuye Sylvia(Computer Number: 514700603) has been approved as partial fulfilment of the requirements for the award of the Master of Science degree in Pathology (Haematology) at the University of Zambia.

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## Abstract

Spontaneous abortion in Zambia is defined as non-induced embryonic death or passage of products of conception before 28 weeks' gestation. Placental insufficiency as a result of thrombi lodging in the uteroplacental bed is considered to be a cause of pregnancy loss. Circulating microparticles are minute sized particles associated with prothrombotic nature and have been found to be increased in the uteroplacental circulation in pregnancy, as such microparticles may be directly or indirectly associated with pregnancy loss. With abortion cases accounting for approximately 80% of cases presenting at the Obstetrics and Gynaecology emergency ward at The University Teaching Hospital, the objective of this study was to determine if circulating microparticles are associated with the risk of recurrent abortion.

A total of 18 women with two or more recurrent abortions were enrolled in a case-control study and compared with 55 parous women as controls. After undergoing counselling performed by a trained midwife 24 hours after pregnancy loss or birth, a consent form was signed thereafter a questionnaire completed and 3mls of blood collected in a citrated container. Microparticles were measured in the isolated platelet poor plasma by flow cytometry using fluorescent annexin V-fluorescence isothiocyanate conjugate.Microparticle levels in cases was compared to controls using unpaired t-test and logistic regression used to determine the parameters of the questionnaire associated with abortion. Increased microparticle levels for both cases and controls was defined as more than 2 standard deviation above the mean of controls. The mean in this study was found to be 202/µl and was identified in 4 of the 18 women with recurrent abortions (22%), as compared to 2 of the 55 parous women (4%), t(20)=2.49, P=0.022, 95% CI 182.20-16.09.

The increased levels of microparticles in the peripheral circulation of women with two/more unexplained pregnancy losses supports emerging evidence that microparticles have a role to play in abortion, however, more studies need to be carried out to confirm these findings.



Key Words: Recurrent Abortion, Circulating Microparticles, Social Demographics.

## Dedication

This work is dedicated to my supportive parents Mr Cosmos Chibuye and Mrs Doris Chibuye.

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## List of Abbreviations

| | |
|---|---|
| **CD** | Cluster of Differentiation |
| **CI** | Confidence Interval |
| **FACS** | Fluorescence Activated Cell Sorter |
| **FITC** | Fluorescein Isothiocyanate Conjugated |
| **MPs** | Microparticles |
| **MSc** | Master of Science |
| **OR** | Odds ratio |
| **PAC** | Post-Abortal Care |
| **PPP** | Platelet Poor Plasma |
| **PS** | Phosphatidylserine |
| **RCOG** | Royal College of Obstetricians and Gynaecologists |
| **RSA** | Recurrent Spontaneous Abortion |
| **SCC** | Side Scatter |
| **SD** | Standard Deviation |
| **TF** | Tissue Factor |
| **UTH** | University Teaching Hospital |
| **vWF** | Von Willebrand factor |

# Chapter 1: Introduction

## 1.1 Background

According to the Royal College of Obstetricians and Gynaecologists (RCOG) Green-top Guideline No. 17, an abortion can be defined as the spontaneous loss of a pregnancy before the fetus has reached viability at 24 weeks (Regan et al., 2010). This includes all pregnancy losses from the time of conception until 23 completed weeks of gestation. In North America, an abortion is considered to be pregnancy loss before the fetus has reached viability at 20 weekswhilst in Zambia it is considered to be a pregnancy loss before the fetus has reached viability at 28 weeks. The American Society for Reproductive Medicine (2008) defines recurrent abortions as two or more failed pregnancies, which have been documented by either ultrasound and/or histopathological examination.

Advances in technology combined with contributions from authors in trying to understand mechanisms that lead to recurrent pregnancy loss has led scientists to believe that, a heterogeneous group of small membrane-coated vesicles with a diameter of 0.1-1μm called circulating microparticles (MPs) could be responsible. These MPs represent sub cellular elements for cell signaling and intercellular communication in inflammation and thrombosis (Distler et al., 2005). They are markers of cell activation associated with various prothromboticstates (Thaler et, al. 2011), and are released from cell membranes upon activation or apoptosis. Evidence has shown that MPs due to their thrombogenic potential and increased circulation through the uteroplacental circulation in pregnancy, contribute to thrombosis in the uteroplacental bed thereby leading to placental insufficiency and ultimately resulting in pregnancy loss (Jy et al., 2010).

Over time scientists have found that surfaces of circulating microparticles display proteins from their parental cells (Mause et al., 2010) which enable identification of their cellular origin. Phosphatidylserine (PS) is also expressed on their surface thus exhibiting prothrombotic features by providing a catalytic surface which promotes

assembly of procoagulant proteins and stimulates coagulation reactions (Castellana et. al, 2009). Microparticles have receptors for both collagen and Von Willebrand factor (vWF) (Owens et, al. 2010) and surfaces of all PS positive MPs have 50-100-fold increased procoagulant ability than the same surface area of an activated platelet as illustrated in figure 1. Procoagulant and/or inflammatory response on the endothelial surface is due to increased expression of adhesion molecules (Sinauridze et, al. 2007).

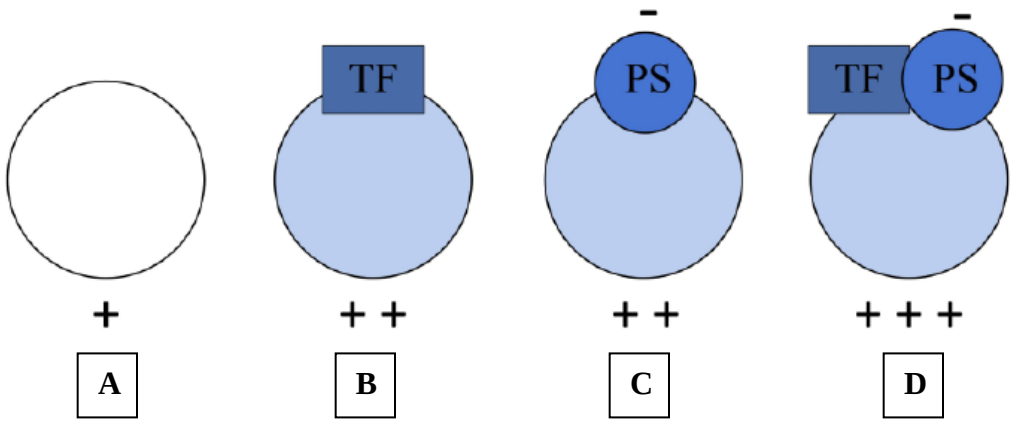


**Figure 1** Increasing procoagulant activity of Microparticles.

(A) The parental cell is decisive to whether the MP is bearing tissue factor (TF) (B) MP with TF. (C) MP with phosphatidylserine (PS) and does not express TF. (D) MP showing both TF and PS is the most procoagulant. Procoagulant activity increases to the right. The negative bars over PS (-) indicate that the surface is negatively charged. (Key., 2010), figure by Dr. Nigel Mackman.

Studies of this nature have not been done in Zambia. Results from this study aim to assist obstetricians and gynaecologists in identifying women whose pregnancy loss may be associated with increased levels of circulating microparticles. These women would be considered to be high risk and needing close monitoring.

## 1.2 Statement of the Problem

One of the commonest complications of pregnancy is abortion. Despite a wide range of studies, there is no apparent cause found in about 60-70% of abortion cases (Alijotas-Reig et al., 2013).Approximately 10-15% of all clinically recognised pregnancies end up in abortion. About 2% experience two consecutive losses and 0.1-1% of women experience three consecutive losses (Toth et al., 2009). Risk of abortion at less than 6 weeks of gestation ranges from 22-57%, at 6-10weeks it declines to 15% and is at 2-3% after 10 weeks of gestation (Toth et al., 2009). The risk of having an abortion increases with maternal age and parity. At less than 35years the risk is at 19% and increases to 47% in those over 35 years (Distler et al., 2005). Prospective and retrospective studies have both shown that the risk of a further abortion increases after each successive pregnancy loss, reaching 45% after three consecutive pregnancy losses (Nybo et al., 2000).

The University Teaching Hospital receives approximately 500 abortion cases monthly, which accounts for 80% of cases presenting at the Obstetrics and Gynaecology emergency ward.

### 1.3 Justification of the Problem

Abortions induce pronounced emotional responses such as anxiety, depression, denial, anger, marital disruption and a sense of loss and inadequacy (Yi-xiang et al., 2016). Women with sporadic and recurrent abortion show high levels of pregnancy-related fear and state of anxiety (Fertl et. al, 2009). A third of women who attended a specialist clinic in Denmark were identified as clinically depressed and one in five showed levels of anxiety similar to those in the psychiatric outpatient population. (Flaumenhaft, 2006). Therefore, research into improved diagnosis and development of new treatment strategies is essential.

A study in China investigated the recurrent abortion associated psychological effects and sexual functions of Chinese men whose partners had experienced a history of pregnancy loss, found men whose partner had experienced a pregnancy loss had higher levels of anxiety, depression as well as erectile dysfunction. This showed that recurrent abortion not only affects women but men as well (Yi-xiang et al., 2016).

Identifying women at risk of abortion could help obstetricians and gynaecologists to better manage these women thereby reducing the incidence rate of abortion.

## 1.4 Literature Review

Abortion is the most common complication of early pregnancy (Doubilet et al., 2013). The frequency tends to reduce with increasing gestational age. The incidence of abortion in clinically recognised pregnancies upto 20 gestational weeks is 10 to 15%. The incidence reduces to about 2% in women who experience two consecutive losses and 0.1-1% in women with three consecutive losses (Toth et al., 2009).

### 1.4.1Microparticle formation

The mechanisms leading to the formation of microparticle in vivo has not yet been established. However, knowledge on microparticle formation in vitro is available, originating from experiments performed on isolated or cultured cells (Burnier, 2009). At rest the cell membranes' asymmetric distribution of the lipid bilayer is maintained by three enzymes: flippase, floppase and scramblase. Flippase specifically transfers the PS from the outside to the inside of the cell membrane whilst, floppase transports phospholipids from the inside to the outside. Scramblase promotes bidirectional redistribution across the lipid bilayer and is inactive when cells are in resting state (Bevers, 1999).

Microparticle formation is initiated during cell activation, apoptosis or senescence due to a significant increase of calcium released by the endoplasmic reticulum of the cell that releases the MP (Hugel, 2005). Calcium inactivates the flippase and activates the flopppase and sramblase leading to loss of phospholipid asymmetry. This results in membrane budding and shedding of MPs enriched with PS on the surface (Hugel, 2005).

### 1.4.2 Relationship between microparticles and abortion

Carp et al (2003) questioned whether MPs particularly of endothelial origin would cause abortions or if they were just simply a byproduct of embryonic demise.

Toth et al (2008) in a German study investigated the relationship between circulating MPs and systemic coagulation activation in recurrent spontaneous abortions (RSA). It was found that the number of annexin V binding MPs was nearly similar in both

cases and controls, this lead them to believe that increased number of circulating MPs are either only indirectly associated with coagulation during pregnancy of RSA patients or affect abortion via mechanisms that are independent from that of hypercoagulability.

In a Korean study, structural anomalies were found in 10 of 19 missed abortions examined by means of transcervical embryoscopy (Philipp and Kalousek, 2001). Embryonic structural anomalies have been shown to be associated with increased cytokines such as transforming growth factor β, transforming growth factor α and apoptosis (Rai and Regan 2006). This suggests that embryonic death occurred due to genetic or structural anomalies, proinflammatory cytokines or inappropriate apoptosis. However, these mechanisms ultimately result in microparticle formation and thrombosis and this has been hypothesised as the trigger of pregnancy loss.

Greer (2001) suggested that various complications of pregnancy, including abortion, may be associated with procoagulant changes, rather than congenital or acquired thrombophilias in particular.

A study in France showed that MPs are elevated in normal pregnancy (Bretelle et al., 2003), indicative of an ongoing process of cell activation.

Aharon et al (2009) in an American study demonstrated that circulating MP from healthy pregnant women had a higher procoagulant activity than circulating MP from that of non-pregnant women. The article also revealed tissue factor/tissue factor pathway inhibitor ratio to be higher in woman with normal pregnancies as compared to that of non-pregnant women.

Carp et al, (2004) in a case-control study in Israel evaluated a cohort of cases with recurrent abortions and found that there was a significant relationship between poor obstetric outcome and MPs. This lead them to conclude that a proportion of women with recurrent abortions have elevated endothelial cell MPs thus suggesting that endothelial activation and/or damage might be associated with the pathogenesis of abortion.

Similarly, Laude et al., (2001) in a study carried out in France assessed the prevalence of circulating MPs in 74 women with a history of unexplained pregnancy loss and 50 controls. The cases were separated into two groups the first group being early pregnancy loss (three or more RSA at or before the $10^{th}$ post menstrual week) and the second group being late pregnancy loss (one death at or after the $10^{th}$ post menstrual week). 29 women from the first group and 12 women from the second group gave a total of 41 of the 74 women (48.6%) who showed high levels of MPs, as compared to 3 of 50 (6%) in the control group. These findings lead Laude et al to conclude that MPs are a promising marker for understanding recurrent abortions.

Studies that have been carried out to investigate the relationship between MPs and obstetric complications include Pre-eclampsia and Intrauterine Growth Restriction (Van Wijik et al., 2002) which revealed a strong association. This study intends to investigate the relationship between microparticles and recurrent abortion in order to identify women at high risk of abortion and in need of close monitoring.

### 1.4.3 Socio-demographics associated with abortion

Established risk factors for abortion include increased maternal age (De la Rochebrochard and Thonneau 2002), history of miscarriage and infertility (Axmon and Hagmar 2005). However, the relationship between age, parity, infertility and previous pregnancy loss is still not entirely understood. Behavioural and social risk factors include alcohol consumption,smoking, and caffeine intake are the main examples (Rasch 2003; Bech et al.,2005).The few studies done on vitamin supplementation support a protective effect (Bailey and Berry 2005).There is an increasing interest in the role that stress and emotional well-being play in pregnancy. Paternal age is reasonably well established (Nybo et al., 2004), current evidence relating the effect of other paternal factors, including paternal occupation, and alcohol drinking and smoking prior to conception, to risk of miscarriage is limited (Maconochie et al., 2006) and warrants further investigation.

## 1.5 Research Question

Are circulating microparticles a risk factor for recurrent abortions in women at the University Teaching Hospital?

## 1.6 Objectives

### 1.6.1 General Objective

To study the relationship between circulating microparticles and recurrent abortion at the University Teaching Hospital.

### 1.6.2 Specific Objectives

i. To detect circulating microparticles in women with recurrent abortions at the University Teaching Hospital using flow cytometry.

ii. To detect circulating microparticles in control parous women with no history of abortion at the University Teaching Hospital using flow cytometry.

iii. To determine the association between circulating microparticles in women with recurrent abortions and parous women with no history of abortion.

iv. To determine the socio-demographics associated with recurrent abortions.

# Chapter 2: Methodology

## 2.1 Study Design

This was a case control study.

## 2.2 Study Setting

The study was carried out at The University Teaching Hospital, Department of Obstetrics and Gynaecology.

Cases where recruited from C03 ward, whilst controls where recruited from B03 ward.

## 2.3 Study Population

Study population included women with two or more pregnancy losses before viability, taken as 28 weeks, that meet the eligibility criteria.

## 2.4 Cases and Controls

A case was defined as a woman>18 years of age that had suffered two or more recurrent abortions with unknown possible cause attending the Obstetrics and Gynaecology emergency ward at The University Teaching Hospital between May 2016 to July 2016. Only women whose pregnancy loss had no known cause after laboratory investigations were included in this study, such women were approached 24 hours after the pregnancy loss by a trained midwife to participate in the study. Women that were willing, first underwent individual counselling and were required to sign a consent form and to help the midwife complete a questionnaire after which 3ml of blood was collected. Altogether 18 cases were enrolled.

Hospital based controls were drawn from women>18years of age having at least two live births and no history of pregnancy loss.Women were approached 24 hours after the birth during their postnatal in B03 ward where women who met the criteria and were willing to participate in the study were talked to by the research team and required to sign a consent form and a blood sample collected. In total 55 controls were enrolled.

Selection of both cases and controls was by purposive sampling. All women that met the criteria and were willing to participate in the study where included. Due to a limited number of cases (two or more consecutive pregnancy losses) as compared to the plentiful supply of potential controls (two or more live births), in order to increase the statistical power of the study more controls than cases were enrolled in a 3:1 ratio giving a sample size of 18 cases versus 55 controls.

### 2.5 Ethical Approval

Ethical approval was sought from The University of Zambia - Biomedical Research Ethics Committee. Consent was granted by the University Teaching Hospital administration and permission to collect samples from the Obstetrics and gynaecology wards was sought from the Head of the Department of Obstetrics and Gynaecology at the University Teaching Hospital (UTH). Written informed consent was obtained from all the participants in the study.

### 2.6 Blood Processing

3ml of blood was collected from cases and controls a day after pregnancy loss or child birth respectively and gently mixed in a sodium citrate vacutainer. The blood samples were isolated by a two-cycle centrifugation process in order to obtain platelet poor plasma. The first centrifugation cycle was for 10 minutes at 1500rpm and the second was at 1300rpm for 5 minutes. The platelet poor plasma was stored at -35$^{o}$C until analysis and the remaining sample was discarded.

## 2.7 Circulating Microparticles Assessment

The reagents were brought to room temperature before the procedure.

30µl platelet poor plasma was incubated for 30 minutes at room temperature in the dark with 10µl Annexin V fluorescein isothiocyanate (FITC). After incubation was complete, samples were diluted in 500µl of annexin V binding buffer solution (1:10 in distilled water). To express microparticle counts as absolute numbers per microliter of plasma, 30µl of counting beads with an established concentration close to 1000beads/µl was added to each sample (CountBright™ absolute counting beads, Life Technologies, Canada). The sample was vortexed at a low frequency for 10 seconds and the number of fluorescence-positive microparticles in the stained samples quantified by the Becton Dickinson FACS Calibur (Fluorescence Activated Cell Sorter) after 10, 000 events.

## 2.8 Flow Cytometric Analysis

Standardisation of microparticles analysis was done using the BD FACS Calibur using a blend of monodisperse fluorescent beads (Megamix, Biocytex, Marseille, France) having three diameters (0.16µm, 0.2µm and 0.5µm) as shown in Figure 2. The beads were used to increase sensitivity by assigning size gates in which the microparticles could be detected after adjusting threshold and voltages of FITC and SCC.

The total number of microparticles was calculated using the formula:

$$\text{MP/ µl} = \frac{\text{Number of events in region containing MP}}{\text{Number of events in absolute count bead region}} \times \frac{\text{Number of beads per test*}}{\text{Test volume}}$$

where * is provided by the manufacturer.

**(A)** **(B)**

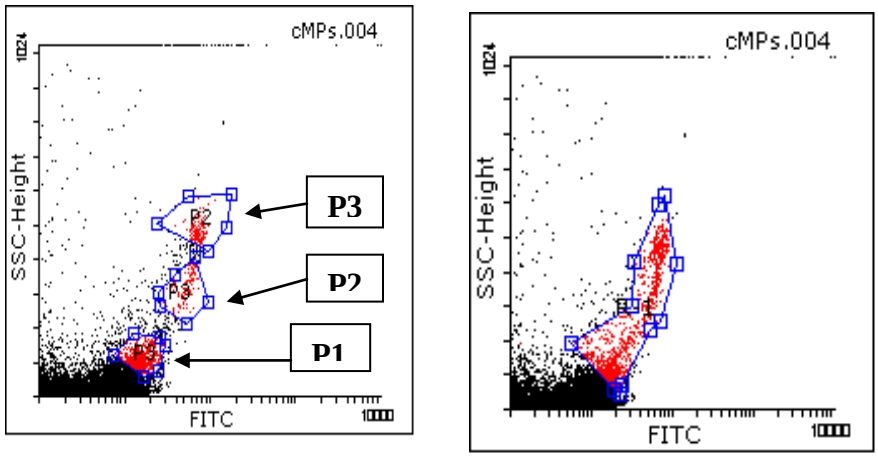


**Figure 2.** FITC and SCC Microparticle gate using Megamix beads. (A) Gating for megamix beads: region P1: 0.16µm, region P2: 0.2µm, region P3: 0.5µm. (B) Microparticle gate 0.16µm-0.5µm.

### 2.9 Data Collection Tool

A questionnaire was used in order to collectinformation on socio demographics of both cases and controls.

### 3.0 Statistical Analysis

An increased level of MPs in both cases and controlswas defined as a level >2 standard deviation (SD) from the mean of control women (Patil et al., 2013). MP levels in cases were compared with controls using the unpaired t-test, where statistical significance was assumed at $P< 0.05$, 95% confidence interval (CI).

Logistic regression was used to determine parameters in the questionnaire associated with abortion.

Data was analysed using SPSS version 16 software.

## Chapter 3: Results

### 3.1 Flow Cytometry

MP levels were determined in a total of 73 women, 18 cases and 55 controls. Levels that were >2SD from the mean of control women were the cut-off for increased levels of MPs in both cases and controls. The cut off value was found to be at 202MPs/µl. A scatter plot of MP levels in both cases and controls is demonstrated in Figure 3.MP levels ranged from 0 to 495/µl in cases and 0 to 451/µl in controls. Four of the 18 cases had an increased number of MPs 22(%) and 2/55 controls 4(%) had increased levels, P=0.022. Figure 4 illustrates the mean distribution of microparticles in cases and controls with cases have a higher mean than controls.

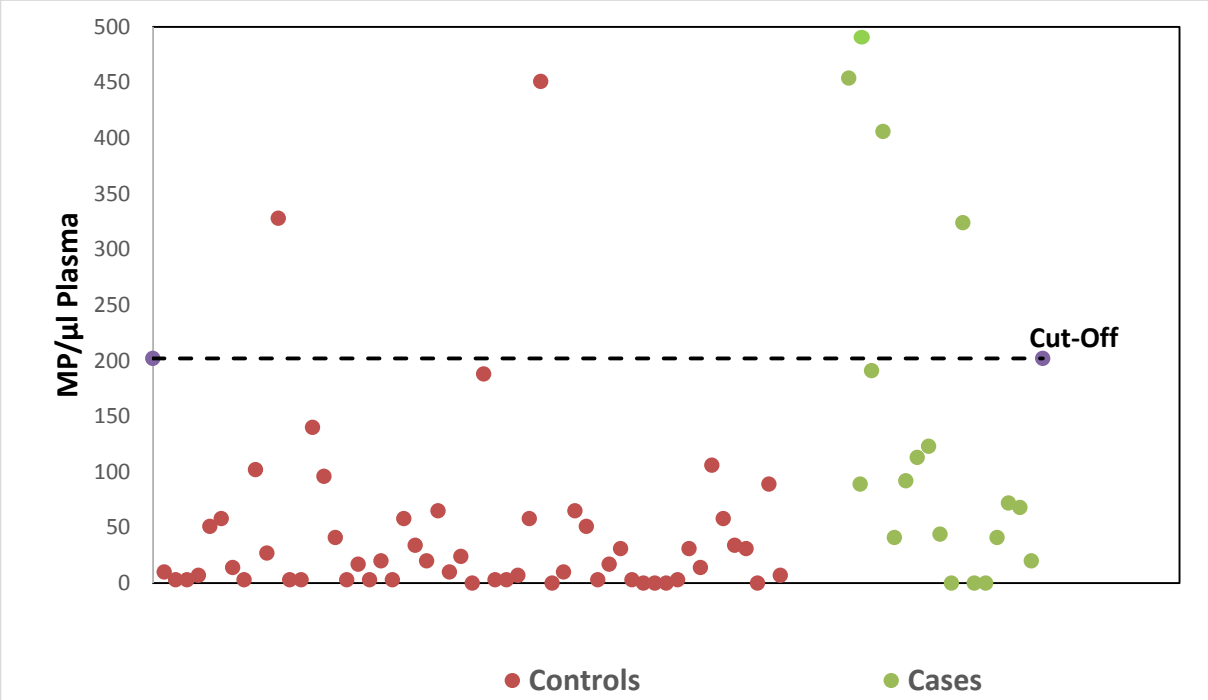


**Figure 3.** Distribution of micropaticles in cases and controls.

MPs = Microparticles

Among women at the University Teaching Hospital (N=73) there was a statistical significance between the two groups of women, Cases (mean=142.94, SD=162.594) and controls (mean=43.80, SD=78.977), t(20)=2.49, P=0.022, 95% CI 182.20-16.09.

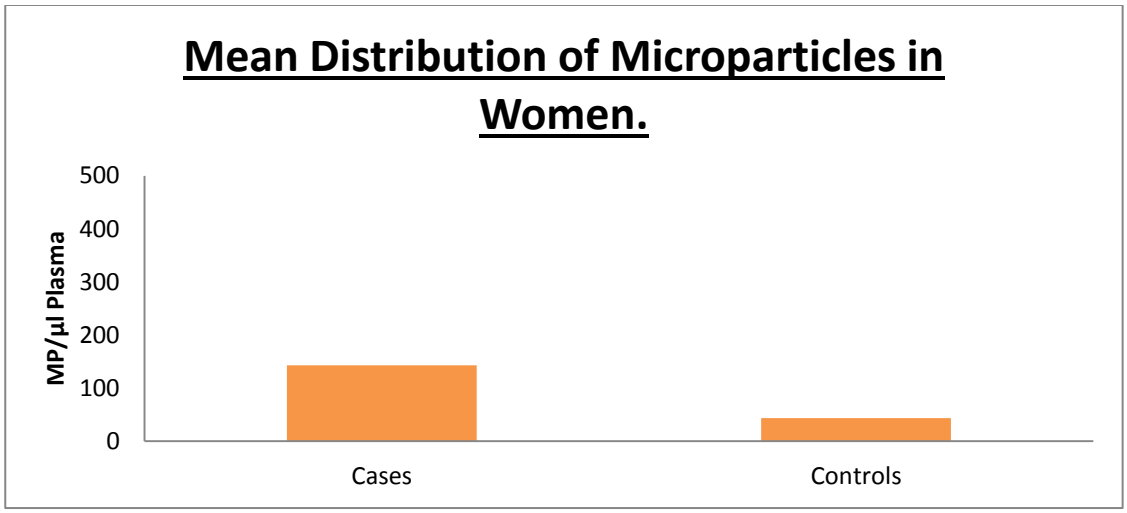


**Figure 4.** Mean distributionof Microparticles in cases and controls.

### 3.2 Questionnaire

Characteristics of the study participants are demonstrated in table 1

**Table1.** Characteristics of study participants

| Characteristic | Controls[no. (%)] | Cases[no. (%)] |
|---|---|---|
| **Maternal age** <18 | 1(6) | 9 (16) |
| 19-29 | 2(11) | 25 (45) |
| 30-34 | 4(23) | 19 (36) |
| 35-39 | 6 (33) | 2 (16) |
| >40 | 5(27) | 0 (0) |
| **Body mass index**<18.5 | 2 (11) | 4 (7) |
| 18.5-24.0 | 1 (6) | 40 (73) |
| 25.0-29.0 | 12 (67) | 10 (18) |
| >30 | 3 (16) | 1 (2) |
| **Paternal age**<18 | 0 (0) | 7 (13) |
| 19-29 | 1 (6) | 23 (42) |
| 30-39 | 5 (27) | 22 (40) |
| >40 | 12 (67) | 3 (5) |
| **Education level**Primary | 12 (67) | 39 (39) |
| Secondary | 4 (22) | 12 (12) |
| Tertiary | 2 (11) | 4 (4) |
| **Fruit &vegetable diet**Yes | 12 (67) | 32 (32) |
| No | 6 (33) | 23 (23) |
| **Caffeine intake**Yes | 7 (39) | 20 (36) |
| No | 11 (61) | 35 (64) |
| **Alcohol consumption**Yes | 18 (56) | 38 (31) |
| No | 8 (44) | 17 (69) |
| **Vitamin supplementation**Yes | 12 (67) | 11 (20) |
| No | 6 (33) | 44 (80) |

Table 2 shows the unadjusted odds ratio for the risk of recurrent abortion in women at The University Teaching Hospital.

Parameters found to be associated with increased risk of recurrent abortion were reduced maternal age (OR 0.26, 95% CI 0.13-0.52) P<0.001, reduced BMI (OR 0.14, 95% CI 0.05-0.41) P<0.001, reduced fruit and vegetable diet (OR 0.78, 95% CI 0.32-1.70) P<0.001, reduced vitamin supplementation (OR 0.13, 95% CI 0.04-0.41) P<0.001 and increased alcohol consumption (OR 1.79, 95% CI 0.06-5.33) P=0.030.

**Table 2.** Unadjusted odds ratio for risk of recurrent abortion.

| Parameter | Odds Ratio | 95% Confidence Interval | P value |
|---|---|---|---|
| Maternal age | 0.26 | 0.13-0.52 | **<0.001** |
| Body Mass Index | 0.14 | 0.05-0.41 | **<0.001** |
| Paternal age | 0.75 | 0.25-2.30 | 0.614 |
| Education level | 0.10 | 0.32-0.31 | 0.478 |
| Fruit and Vegetable diet | 0.78 | 0.32-1.70 | **<0.001** |
| Caffeine intake | 0.90 | 0.30-2.69 | 0.847 |
| Alcohol consumption | 1.79 | 0.60-5.33 | **0.030** |
| Vitamin supplementation | 0.13 | 0.01-0.55 | **<0.001** |

Table 3 shows adjusted odds ratio for the risk of recurrent abortion in women at The University Teaching Hospital.

Parameters found to be associated with increased risk of recurrent abortion were reduced education levels (OR 0.15, 95% CI 0.03-0.87) P=0.035 and reduced vitamin supplementation (OR 0.08, 95% CI 0.01-0.55) P=0.011.

**Table 3.** Adjusted odds ratio for risk of recurrent abortion.

| Parameter | Odds Ratio | 95% Confidence Interval | P value |
|---|---|---|---|
| Maternal age | 0.48 | 0.16-1.44 | 0.194 |
| Body Mass Index | 0.47 | 0.13-1.71 | 0.254 |
| Paternal age | 1.56 | 0.30-8.22 | 0.600 |
| Education level | 0.15 | 0.03-0.87 | **0.035** |
| Fruit and Vegetable diet | 0.61 | 0.38-0.55 | 0.614 |
| Caffeine intake | 3.36 | 0.47-23.90 | 0.227 |
| Alcohol consumption | 1.21 | 0.19-7.82 | 0.842 |
| Vitamin supplementation | 0.08 | 0.01-0.55 | **0.011** |

## Chapter 4: Discussion

Findings in this study showed that women with two or more pregnancy losses had a higher number of microparticles (22%) as compared to control women (4%) who had two or more live births, indicating that microparticles are associated with pregnancy loss P=0.022. Parameters found to be associated with risk of abortion was reduced vitamin supplementation and reduced educational level. There was no association found with maternal age, body mass index, paternal age, fruit and vegetable diet, alcohol and caffeine.

### Flow cytometry

Findings from this study weresimilar to a study by Carp et al (2004) where cases (12.5%) had a higher number of microparticles than controls (2.2%). Laude et al. (2001) carried out a study to investigate the relationship between microparticles and women with unexplained pregnancy losses. A total of 74 women with recurrent abortions was sampled and phosphatidylserine-positive microparticles measured using annexin V-FITC.Forty-one of the74 women had increased levels of microparticles, with 29 (59%) being associated with early pregnancy loss.Similarly, a study by Pasquier et al. (2013) compared microparticle levels of women referred for unexplained pregnancy loss with those of parous women. Women with unexplained pregnancy loss were found to have higher microparticle levels than that of parous women. This study like previous studies that focused on annexin V-FITC positive microparticles also found higher levels of microparticles in women with recurrent abortions as compared to parous women. This study had a lower prevalence (22%) with a sample size of 73 women than that of the study by Laude et al. (2001) that found 59% with a sample size of 74 women. This was because this study collected samples from women 24hrs after either an abortion or a live birth, whilst Laude et al. sampled women 3 months after either a spontaneous abortion or a live birth. Haemostatic changes tend to normalise 3 to 4 weeks after pregnancy loss or birth and as such microparticles have been suspected to equally normalise, thereby setting the

ideal time for collecting samples for microparticle analysis at least 4 weeks after pregnancy loss or live birth. Also, due to the highly thrombogenic nature of microparticles, they tend to get consumed through the processes of excessive clotting initiation and activation in the placental bed and thus most of the microparticles would be trapped in fibrin deposits explaining the lower prevalence of microparticles in our study.

Pregnant mice injected with artificial phospholipid vesicles containing phosphatidylserine, induced thrombosis in the placental bed and reduced birth weight, thrombosis as well as necrosis was also observed. This supported a possible role for (phosphatidylserine-positive) microparticles in disturbed pregnancy (Sugimura et al., 1999). Overtime studies have shown that 29-60% of recurrent abortions are due to chromosomal aberrations (Carp et al., 2003). However, embryonic structural anomalies have been linked to various cytokines as well as apoptosis. These mechanisms inadvertently result into microparticle formation and thrombosis thereby triggering pregnancy loss.Utility of annexin V in flow cytometry applications is derived from its' selective affinity for negatively charged phospholipids displayed on membranes of various cells.

**Questionnaire**

Results from the questionnaire showed that vitamin supplementation particularly folic acid and vitamin B12 and a fruit and vegetable diet conferred a protective effect against abortion, controlling for all parameters in the model. Fruit and vegetable diet was not statistically significant. Studies that found it statistically significant defined diet as fruit, vegetable, meat and dairy (Maconochie et al., 2007) whilst this study defined diet as fruit and vegetable only. Thus, women who did not necessarily have a fruit and vegetable diet had meat and dairy, explaining why fruit and vegetable diet was not statistically significant in this study. Vitamin supplementation is crucial in the early stages of foetal life as it is required for synthesis of DNA and red blood cells as well as the production of the myelin sheath around nerves. Lack of vitamins could

lead to genetic abnormalities such as Spina Bifida and may ultimately result in pregnancy loss. PerWardlaw and Kessel (2002) excessive alcohol consumption is associated with low birth weight babies, spontaneous abortion, developmental abnormalities as well as foetal malformations. Other harmful effects of alcohol consumption on the infant can include low birth weight, a small head and low birth length (Williamson, 2006). A UK study found low body mass index (BMI) to be linked to a 75% increase in risk of abortion (Maconochie et al., 2007). This study found a 53% increase in risk,considering all the parameters in the model. Low BMI could be a marker of low fruit and vegetable diet and low vitamin supplementation (Axmon and Hagmar, 2005) thereby resulting in pregnancy loss. Garcia et al (2002) and Maconochie et al (2007) in different studies found educational levels of women to have no association with the risk of abortion. In this study, lower educational levels were linked to risk of abortion and this was statistically significant. This could be due to indirect effects on food choices and feeding patterns of family members as well as lack of understanding of nutrition and food aspects (Walravenet al., 1997). Most women in this study had attained only primary level education. Reduced maternal age and increased paternal age wasfound to be associated with risk of abortion despite not being statistically significant which was similar to Stewart et al (2007) in a study in Nepal. Findings in this study supported by Rasch (2003), found no association between caffeine intake and abortion. More work on the association of caffeine on pregnancy loss needs to be investigated.

# Chapter 5: Conclusion and Recommendations

## 5.1 Conclusion

This study showed increased levels of microparticles in peripheral circulation of women with two or more unexplained pregnancy losses regardless of it being an early loss or a late loss, thus supporting emerging evidence that microparticles have a role to play in abortion. This shows a promising approach to the use of microparticles as markers for risk of recurrent abortion. Studies are being done that are investigating administration of anticoagulants to counteract the effects of circulating microparticles, as a measure to manage women that affected.

Low educational levels were found to be associated with increased risk of abortion whilst vitamin supplementation was found to confer a protective effect against abortion.

## 5.2 Strengths and Weaknesses

One of the strengths of this study is its novelty; no work has been published on circulating microparticles in Zambia. Hence, this can be used as a baseline study for future works.

One of the weaknesses is that the study sample size that was calculated and adopted was similar to other studies but could be too small to make generalised inferences, however, it has yielded some very important information which can be used to do further studies.

## 5.3 Future Works

Microparticles should be analysed at different time intervals from the time of pregnancy loss in order to establish the best time to sample blood for microparticle analysis. Studies to investigate the effects of anticoagulants on levels of

microparticles should be done in order to better understand how best unexplained pregnancy losses can be handled.

Studies focusing on socio-demographics associated with risk of recurrent abortion should be done in order to confirm findings from this study as well as to cover more parameters.

### 5.4 Recommendations

A larger study not restricted to the University Teaching Hospital would need to be carried out to generate more representative results as a sample size of 73 does not give a true picture of recurrent abortion and its' burden in our country. However, the information from this study can be used as a stimulus to start pushing for the use of microparticle analysis as a routine test for determining the risk of recurrent abortion.

Vitamin supplementation should be encouraged as it was found to confer a protective effect. Low education levels were associated with increased risk of abortion and as such ways of reaching out to women in both rural and urban areas need to be investigated so as to help equip these women with knowledge on risks associated with abortions.

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## Appendices

### Appendix A

### Participant Information Sheet

My name is Chibuye Sylvia, I am carrying out a research that will contribute to my completion of my Master of Science degree from The University of Zambia.

About the Study

An abortion is loss of a pregnancy before reaching 28 weeks and a recurrent abortion is two or more failed pregnancies. 60-70% of abortion cases have no cause, however, microparticles have been shown in different articles to be found in large numbers in women with recurrent abortions. The study aims to find out if these microparticles can be used to identify women who are at risk of recurrent abortions, this information could lead to further research aimed to reduce the risk of recurrent abortions.

Participating in the Study

You are being asked to take part in this study because you a woman who has either had two or more recurrent pregnancy losses or a woman who has given birth previously to two or more live births. You are not going to be forced to take part. If you decide to take part and you decide to stop, you can do so without any reason. If you decide to take part in this study, it will only take not more than 20 minutes of your time that will also include individual counselling and if you do not feel like answering any/some of questions you will be forced to give an answer. You will be asked to sign a consent form, that will allow the researcher to take 3ml of your blood to be used in this study. After the study your blood sample will be thrown and it will be burned according to hospital regulations.

If you no longer want to take part in the study your personal information will be destroyed and will not be used in the study, this will not affect the type of care you will receive from the staff at UTH.

Benefits for Participation

Information for this study will help find out if circulating microparticles can put a woman at risk of having a recurrent abortion and also help doctors manage these patients.

Problems with the Study

No problems are being expected to arise in this study.

Participant Confidentiality

After the midwife has collected all the information only the researcher will have access to the information and the researcher has used this information all forms containing the participants information will be burnt to avoid other people gaining access to this information.

Contact Details

In case you have any more questions about this study at any time, please feel free to contact any of the numbers below.

**Chibuye Sylvia**
(The Researcher)
Mobile: +260 953670958
Email Address: mwenyachibuye@yahoo.com

**The Chairperson**
The University of Zambia-Biomedical Research Ethics Committee
Telephone: 260-1-256067
Email Address: unzarec@zamtel.zm

**Appendix B**

**Participant Informed Consent Form**

Study title: **"Circulating Microparticles: a risk factor for recurrent abortion in women at The University Teaching Hospital, Lusaka, Zambia."**

I confirm the following;

- I have read (or been read to) and understand the information sheet for this study and I have asked questions where I did not understand.
- I understand that I am not being forced to take part in this study and I can stop at any time.
- I agree to let the researcher use and share the health information and other information she will get from this study and I agree to give 3ml of my blood for use in this study.

**Declaration by Participant:**

I hereby consent to take part in this study willingly.

Participant's signature: _____ Age _____

Thumb Print

**Declaration by researcher taking consent:**

I have given an explanation of the study to the participant, and have answered the participant's questions to the best of my ability.

I believe that the participant understands the study and has given informed consent to participate**.**

**Researcher's signature:_____**

**Appendix C**

**Questionnaire**

Participant number_____

- **What is your weight_____ height _____**

**BMI**

0. <18.5 (underweight)

1. 18.5-24.9 (normal)

2. 25.0-29.9 (overweight)

3. >30.0 (obese)

- **How old are you?**

0. less than 18 years old.

1. 19-29 years old.

2. 30- 34 years old.

3. 35-39 years old.

4. above 40 years old.

- **How old is/was the father of the child?**

0. less than 18 years old.

1. 19-29 years old.

2. 30- 39 years old.

3. above 40 years old.

- **What is your level of education?**

0. Primary education (Grade 1 - Grade 7) .

1. Secondary education (Grade 8 - Grade 12).

2. Tertiary education (College/University).

- **Did you take any vitamin supplements during your pregnancy (Vitamin $B_{12}$ and folic acid)?**

0. No.

1. Yes.

- **How can you describe your diet (Fresh fruits and Vegetables) during your pregnancy?**

0. No.

1. Yes.

- **How can you describe your caffeine consumption during your pregnancy (coffee, energy drinks like red bull etc)**

0. No.

1. Yes.

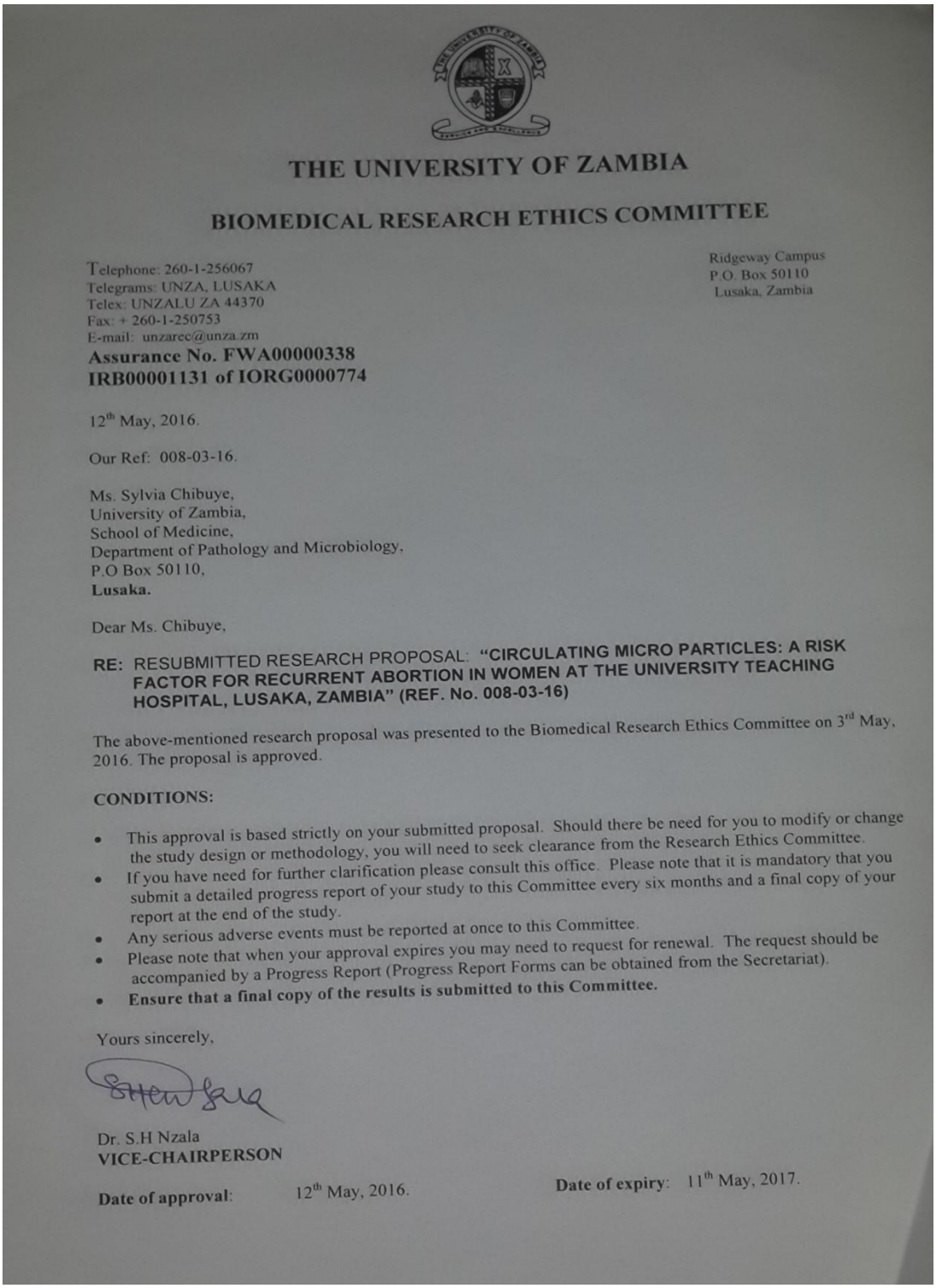
- **Did you consume alcohol during your pregnancy?**

0. No.

1. Yes.

## Appendix D

## UNZABREC Clearance Form



THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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Lusaka, Zambia

**Assurance No. FWA00000338**
**IRB00001131 of IORG0000774**

12th May, 2016.

Our Ref: 008-03-16.

Ms. Sylvia Chibuye,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P.O Box 50110,
**Lusaka.**

Dear Ms. Chibuye,

**RE:** RESUBMITTED RESEARCH PROPOSAL: **"CIRCULATING MICRO PARTICLES: A RISK FACTOR FOR RECURRENT ABORTION IN WOMEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA" (REF. No. 008-03-16)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 3rd May, 2016. The proposal is approved.

**CONDITIONS:**

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

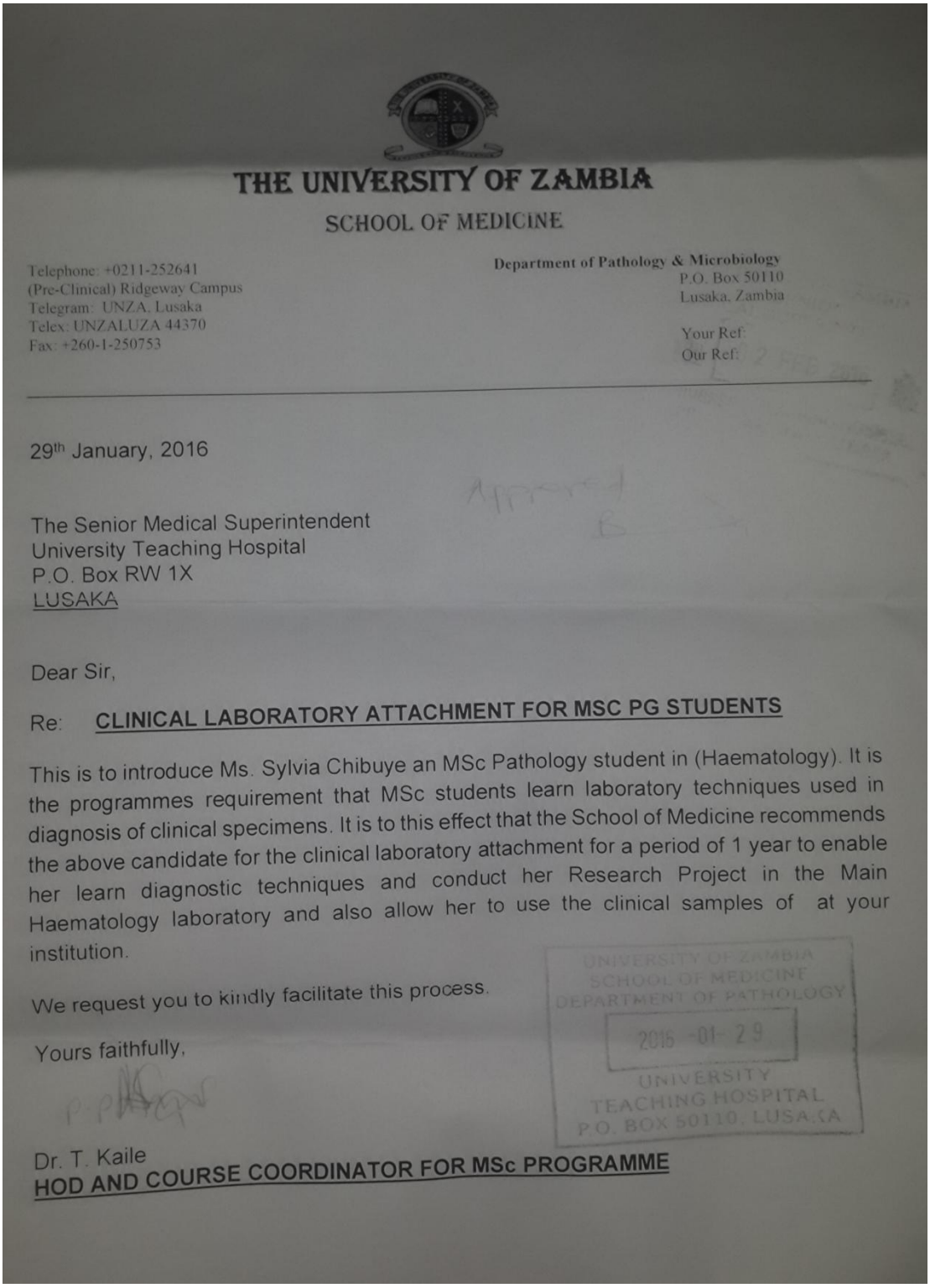
Yours sincerely,

Dr. S.H Nzala
**VICE-CHAIRPERSON**

**Date of approval**: 12th May, 2016. **Date of expiry**: 11th May, 2017.

## Appendix E

## Letter of Authority to conduct research



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Department of Pathology & Microbiology
P.O. Box 50110
Lusaka, Zambia

Your Ref:
Our Ref:

29th January, 2016

The Senior Medical Superintendent
University Teaching Hospital
P.O. Box RW 1X
LUSAKA

Dear Sir,

Re: **CLINICAL LABORATORY ATTACHMENT FOR MSC PG STUDENTS**

This is to introduce Ms. Sylvia Chibuye an MSc Pathology student in (Haematology). It is the programmes requirement that MSc students learn laboratory techniques used in diagnosis of clinical specimens. It is to this effect that the School of Medicine recommends the above candidate for the clinical laboratory attachment for a period of 1 year to enable her learn diagnostic techniques and conduct her Research Project in the Main Haematology laboratory and also allow her to use the clinical samples of at your institution.

We request you to kindly facilitate this process.

Yours faithfully,

UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY
2016 -01- 29
UNIVERSITY
TEACHING HOSPITAL
P.O. BOX 50110, LUSAKA

Dr. T. Kaile
**HOD AND COURSE COORDINATOR FOR MSc PROGRAMME**