A randomised controlled trial on the effectiveness of melatonin in controlling blood loss during Ceaserean section in a tertiary centre in Southeast Nigeria

ABSTRACT

Background: Caesarean section is a common surgical procedure in Obstetrics. Despite the safety in carrying out caesarean section, it is still associated with blood loss and need for blood transfusion when compared to women who had vaginal delivery. Despite the use of uterotonic such as oxytocin, to prevent postpartum haemorrhage, excessive bleeding still occurs during and after caesarean section. Melatonin has been shown to reduce blood loss, which may be useful in prevention of postpartum haemorrhage. Hence, we aimed to determine the efficacy of Melatonin in reducing blood loss during and after elective caesarean section.

Methodology: This was a double-blind randomised controlled trial. Data analysis was done using IBM SPSS (version 25, Chicago II, USA). Continuous variables were presented as mean and standard deviation (Mean ± 2SD), while categorical variables were presented as numbers and percentages. The categorical variables were analysed using Chi square, while means were compared using t-test and Mann-Whitney U test for non-parametric. A P value <0.05 was considered statistically significant.

Result: Sublingual melatonin reduced blood loss by an average of 76.67ml (0.44%) at elective caesarean section compared with placebo group (955.00ml versus 1031.67ml respectively), however this was not statistically significant (p-value = 0.097). Participants that received blood transfusion were higher in placebo group than melatonin group {29 (17.9%) versus 19 (11.7%)} however, the difference was not statistically significant (p-value = 0.159. Moreover, participants that required additional oxytocic were also higher in placebo group compared to melatonin group {45(27.8%) versus 34 (21.0%)} however, the difference between the two was not statistically significant, (p-value is 0.196). There was no major maternal or neonatal side effect.

Conclusion: This study demonstrated that 8mg sublingual melatonin did not significantly reduce blood loss at elective caesarean section. However, more participants in the placebo group required more oxytocic and blood transfusion. So higher doses and/or repetitive doses should be explored in future researches.

Keywords: Melatonin, blood loss, caesarean section, Obstetrics, Haemorrhage

INTRODUCTION

Caesarean section is one of the most common surgical procedures in Obstetrics[1,2]. Despite the safety in carrying out caesarean section, there increased risk of blood loss[3] and need for blood transfusion when compared to women who had vaginal delivery[4]. The average blood loss during caesarean section is 498ml to 787ml[5]. Postpartum haemorrhage (PPH) is a major cause of maternal morbidity and mortality worldwide (25%) with the highest incidence in developing countries[6,7,8]. In Africa, it causes about 33.9% of maternal mortality[7] and 23.0% in Abakaliki, Southeast Nigeria[9].

Administration of uterotonics, in particular oxytocin is the major component in preventing PPH due to uterine atony, but this can only prevent about 60% of postpartum haemorrhage[11] and a proportion of women will proceed to develop PPH despite this active management[7]. Although oxytocin is the first-line agent to prevent uterine atony during caesarean section, its use alone may sometimes, not be effective for prevention of PPH[13]. Apart from anaphylactic reactions, oxytocin, especially in high doses, has negative inotropic, antiplatelet and antidiuretic effects[13]. All these limits it use effectively.

Additional medications such as ergometrine, misoprostol and tranexaemic acid have been investigated as the alternative and adjuvant therapeutic options for prevention and management of postpartum bleeding[11,14]. However, these drugs cannot be universally administered to patients as they are contraindicated in certain conditions. Melatonin (N-acetyl-methoxytryptamine) plays a direct and integral role in uterine physiology in addition to its centrally mediated effects on reproduction[13] and has been suggested as an adjuvant to oxytocin in prevention of postpartum haemorrhage[13]. It is cheap, easy to administer and readily available with minimal adverse effect, compared to other drugs. In addition, it is stable at room temperature, thereby needs no refrigeration. Therefore, additional biochemical haemostatic effects obtained from the use of pro-haemostatic drugs such as melatonin may go a long way in complementing the effects of oxytocics in the prevention of PPH[11].

Melatonin levels increase in maternal blood, amniotic fluid and urine of pregnant women throughout pregnancy, reaching a peak at term[15]. It is hypothesised that any change in the circulating melatonin level, which is synchronised with the light/dark cycle, is likely to be a major determinant of parturition time in pregnant women[13]. Olcese et al also reported that, melatonin acts through the MTNR1B melatonin receptor that is expressed in the myometrium at late term to synergistically enhance oxytocin-dependent signalling and contractions[17]. Sublingual administration of melatonin (6mg) has been shown to reduce blood loss during caesarean section, although this was not clinically significant and further studies were recommended with higher dose of melatonin[13]. It is on this background we aimed to do this study.

METHODOLOGY

STUDY DESIGN

This was a double blind randomised placebo controlled trial.

STUDY POPULATION

The study was carried out among women undergoing elective caesarean section at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA), and Mile four Hospital Abakaliki, Ebonyi state, Nigeria.

STUDY DURATION

The study lasted for a period of seven months, between 19th May and 15th December 2022.

INCLUSION CRITERIA

Pregnant women at low risk of postpartum haemorrhage at 37-42 weeks gestational age, on admission for elective caesarean section, who gave consent to participate in the study and did not have any contraindication to the use of Melatonin were included in the study.

EXCLUSION CRITERIA

Women who have known allergy to Melatonin, prior history of thromboembolism, with bleeding disorders, history of renal disease, history of liver pathology, with varicose veins at increased risk of deep vein thrombosis, psychotic disorder, chronic anaemia, contraindication to regional anaesthesia, chronic hypertension/preeclampsia/eclampsia, antepartum haemorrhage, risk of primary postpartum haemorrhage such as multiple gestation, previous PPH, polyhydramnios, co-existing uterine masses

SAMPLE SIZE DETERMINATION

The minimum sample size was determined using superiority formula for continuous variables[62].

With it a Sample size of 81 was gotten. This was doubled to increase the power of the study to 162 per group.

RANDOMISATION AND CONCEALMENT

The participants were recruited by simple randomization. The participants were randomised by means of a computer generated random numbers using the software Research Randomizer. Using this software, one hundred and sixty-two numbers were randomly generated from a pool of three hundred and twenty-four numbers (1-324). These were assigned to group A (Melatonin group) while the remaining 162 random numbers were assigned to group B (the placebo group). These numbers were inscribed on a piece of paper with the corresponding group written along with the numbers. These numbers (1-324) were also written on brown envelopes and the pieces of paper inserted into the corresponding envelopes. They were then arranged sequentially from 1 to 324.

Concealment of the drugs was done in the sequentially-numbered opaque sealed envelopes (SNOSE) by a hospital pharmacist without revealing the code to the researcher. All the envelopes were kept in a locker that was made accessible to all the members of the research team.

Participants who meet the inclusion criteria, having signed the informed consent form was given sequential study number and the corresponding numbered opaque sealed envelope was then allocated to the patient.

Group A: Received 8mg of sublingual melatonin during preloading, approximately 20 minutes before spinal anaesthesia. (That is, two tablets of melatonin 5mg and 3mg).

Group B: Received 200mg of sublingual vitamin C during preloading approximately 20 minutes before spinal anaesthesia. (That is, two tablets of 100mg of vitamin C).

BLOOD LOSS ESTIMATION:

a. Haematocrit value: The estimated blood loss was measured using the difference in haematocrit values taken before the surgery and 48 hours after the caesarean section using the formula below.[13]

EBL =Pre-operative Haematocrit – Post-operative haematocrit

Where EBL= Estimated blood loss

Blood loss greater than >1000ml was regarded as excessive bleeding.

b. Visual estimation of blood loss: Blood loss was also estimated by visual method. This is the commonest method of estimating blood loss by obstetricians and anaesthetists. As swab count is a universal method for estimating blood loss at caesarean section, Bose P. and colleagues developed a standardized measure of visual estimation of blood loss using swab count and floor spill[34].

Table 1: Guidelines for visual estimation of blood loss[34]

Small, 10-x10-cm 32 ply swab (maximum saturated capacity)	60 ml
Medium, 30- x 30-cm 12 ply swab (maximum saturated capacity)	140 ml
Large, 45- x 45-cm 12 ply swab (maximum saturated capacity)	350 ml
1-kg soaked swabs	1000 ml
50-cm diameter floor spill	500 ml
75-cm diameter floor spill	1000 ml
100-cm diameter floor spill	1500 ml
Vaginal PPH limited to bed only Unlikely to exceed	1000 ml
Vaginal PPH spilling over from bed to floor Likely to exceed	1000 ml

FOLLOW UP

The participants were monitored during their stay in the hospital. They were seen 4 hours after caesarean section to check for postpartum haemorrhage and any other problem. They were also seen on day one, two and day 3. They were followed up until discharge from the facility. The participants were discharge after 4 to 5 days unless there is reason for longer hospital stay. They were instructed to present to the hospital or reach the researcher or any of the research assistants by phone if they have any unforeseen adverse reaction. And they were seen at the postnatal clinic by the managing team at six weeks postpartum.

OUTCOME MEASURES

Primary Outcome Measure is; quantity of blood loss during caesarean section.

Secondary Outcome Measures are

1. The need for additional uterotonics to control bleeding.
2. The need for blood transfusion during or after the surgery
3. Maternal side effects (nausea, vomiting, headache, skin rash, dizziness, hypotension, bradycardia and maternal death)
4. Neonatal side effect.

STATISTICAL ANALYSIS

Data was collated, tabulated then statistically analysed using IBM SPSS software (version 25, Chicago II, USA). Continuous variables were presented as mean and standard deviation (Mean ± 2SD), while categorical variables were presented as numbers and percentages. Chi-square test (X^2) was used for comparison between groups for categorical variables while student t-test and Man-Whitney U test was used for comparison between groups for continuous variables. A difference with a p-value <0.05 was considered statistically significant.

RESULTS

Over the study duration of seven months, 356 patients were assessed for randomisation into the study. Thirty-two were excluded while 324 were allocated to receive either the melatonin or placebo.

Consort diagram

Figure 1: the flow of patients through the study

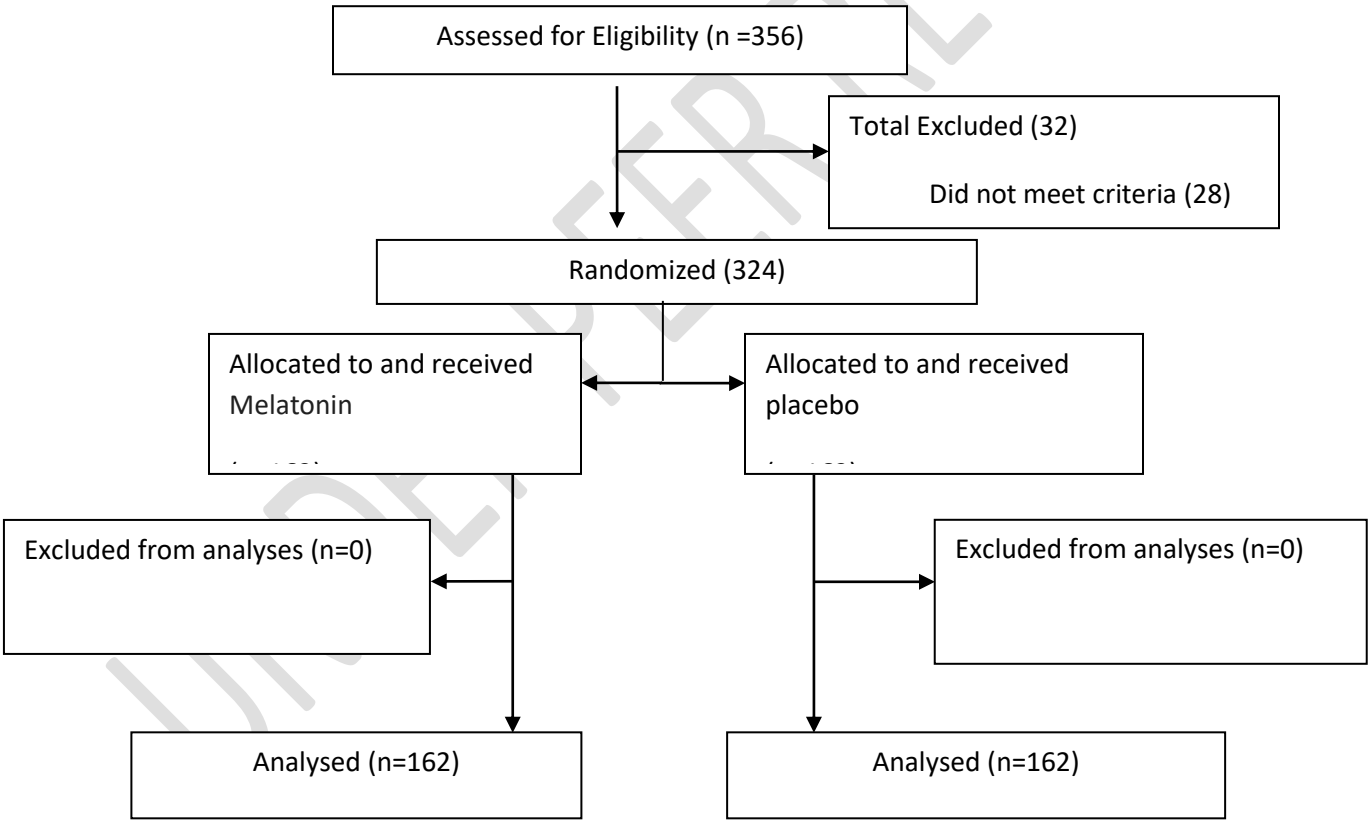

Table 2: Demographic characteristics of the patients and some surgery determinants

Variables	Melantonin (n=162)	Placebo (n=162)	t-test	P-value
Maternal age (years)	30.79±5.90	29.54±6.23	1.851	0.065
Height (m)	1.59±0.06	1.60±0.07	1.377	0.168
Weight (kg)	78.14±12.54	80.01±13.97	1.866	0.206
Body Mass Index (kg/m^2)	30.54±4.68	31.04±5.35	0.896	0.371
Gestational age (weeks)	39.10±1.77	38.81±2.35	1.254	0.211
Parity (median, range)	1 (0-6)	1 (0-6)	11929.00*	0.146

*Mann-Whitney U test used for non-parametric

Table 2 showed no statistically significant difference in all the socio-demographic variables between the two groups.

Table 3: Surgical determinants

Variables	Melantonin (n=162)	Placebo (n=162)	t-test	P-value
Mean duration of surgery (mins)	60.57±12.06	60.37±13.19	0.144	0.887
Fetal birth weight (kg)	3.04±0.58	3.14±0.65	1.418	0.157
APGAR Scores				
First minute	9.47±0.77	9.38±0.70	1.099	0.272
Fifth minute	9.98±0.16	9.94±0.24	1.641	0.102

Table 3 showed no statistical significant difference in the mean duration of surgery in both groups. Also there was no significant difference in neonatal weight and the APGAR scores in the first and fifth minute, (p-value >0.05).

Table 4: Indications for the surgery

Variables	Melantonin (n=162)	Placebo (n=162)	$\chi 2$	P-value
Two previous scars	26(44.8%)	32(55.2%)	0.763	0.385
≥3 previous scars	18(62.1%)	11(37.9%)	1.859	0.173
One previous scar with another indication	28(56.0%)	22(44.0%)	0.854	0.356
Breech presentation	20(54.1%)	17(45.9%)	0.266	0.600
Transverse lie at term	7 (53.8%)	6 (46.2%)	0.081	0.777
Fetal Macrosomia	27(56.2%)	21(43.8%)	0.881	0.348
Intrauterine growth restriction	10(16.7%)	20(83.3%)	3.673	0.055
Others	26(44.1%)	33(55.9%)	1.024	0.314

Table 4 showed that the difference indications for caesarean section between the two groups were not statistically significant p-value >0.05

Table 5a: Intraoperative and postoperative variables

Variables	Melantonin (n=162)	Placebo (n=162)	t-test	P-value
EBL (in ml)	479.01±111.28	481.17±109.06	0.176	0.860
EBL (in %)	5.73±2.47	6.19±2.50	1.673	0.097
Maternal haematocrit				
Preoperative	32.81±2.79	33.25±3.90	1.157	0.248
Post-operative	27.95±2.21	27.32±2.58	2.361	0.019
Change in haematocrit	4.99±2.53	5.61±3.28	1.914	0.058

The estimated blood loss by visual estimation was lower in the melatonin group than placebo group (479.01±111.28ml versus 481.17±109.06ml). The difference between the two was not statistically significant (p = 0.860). More so, blood loss using haematocrit was lower in the melatonin group compared to the placebo group (955.00ml versus 1031.67ml respectively) (p-value 0.097). In other words, melatonin reduced blood loss by 76.67ml (0.44%). There was no significant difference in the pre-operative haematocrit values between both groups p-value was 0.248. But the mean post-operative haematocrit was significantly higher in the melatonin group compare with the placebo group with p-value of 0.019. The change in haematocrit was also higher in placebo group compare to melatonin, but the difference was not statistically significant (p = 0.058).

Table 5b: Intraoperative and postoperative variables and maternal outcomes in the study group and in the placebo group

Variables	Melatonin (n=162)	Placebo (n=162)	P-value	Odd Ratio (95% C.I. OR)
Blood loss (ml)				
≥1000	48 (29.6%)	61(37.7%)	0.158	0.70 (0.44-1.11)
<1000	114(70.4%)	101(62.3%)		
Additional oxytocic				
Yes	34 (21.0%)	45 (27.8%)	0.196	0.69 (0.41-1.15)
No	128(79.0%)	117(72.2%)		
Blood transfusion				
Yes	19 (11.7%)	29(17.9%)	0.159	0.61 (0.33-1.14)
No	143(88.3%)	133(82.1%)		
Side effects				
Headache	33(20.4%)	42(25.9%)	0.292	0.73 (0.43-1.23)
Vomiting	8 (4.9%)	16 (9.9%)	0.136	0.47 (0.20-1.14)
Nausea	4 (2.5%)	5 (3.1%)	1.000	1.34 (0.30-6.09)
PPH				
Yes	48 (29.6%)	61(37.7%)	0.126	2.344*
No	114(70.4%)	101(62.3%)		

*Chi-square test used, PPH – Primary postpartum haemorrhage

Table 5b showed that blood loss ≥1000ml was lower in melatonin group compared to placebo group. The difference between the two was not statistically significant giving the Odd ratio = 0.70, 95% confidence interval = 0.44-0.11 and p-value 0.158. The participants that required additional oxytocic's were higher in placebo group compare to melatonin group but the difference is not statistically significant (p-value0.196). Participants who received blood transfusion were also higher in placebo group than melatonin group but also not statistically significant (p-value 0.159). Difference between the participants that had headache, vomiting and nausea in both groups were not statistically significant (p-values>0.05).There were no other side effects observed in the participants. There was no maternal mortality.

Table 6: Fetal outcome

Variables	Melantonin		Placebo		t-test	P-value
	N	Mean ±SD	*n*	Mean ±SD		
APGAR Score at 1min						
APGAR 7-10	162	9.47±0.77	162	9.38±0.70	1.099	0.272
APGAR Score at 5min						
APGAR ≥ 9	162	9.98±0.16	162	9.94±0.24	1.641	0.102

Table 6 was not statistically significant.

DISCUSSION

This study showed that, pre-operative administration of 8mg of sublingual melatonin reduced blood loss during caesarean section by 76.67ml (0.44%). This might be due to the fact that, melatonin augments oxytocin activity and enhances myometrial cell contractions leading to reduce blood loss[12,16]. The second possible reason is that, melatonin prevents high blood pressure and excessive haemorrhage by reducing anxiety[13]. This finding was higher than 68.33mls (0.41%) by Khezri et al[13] and 55ml (0.33%) by Javadi et al[54]. This difference may be due to the difference in sample size and dosage of melatonin. While Khezri et al and Javadi et al had smaller sample sizes (40 and 70 respectively) the sample size of the current study was 162 per group. Also, Javadi et al study was among pregnant women that delivered vaginally while ours was among women that delivered by elective caesarean section. Also, their haemoglobin estimation was done 24 hours postpartum while our postpartum haematocrit was done after 48 hours. Moreover, Khezri et al use lesser dosage of melatonin, 6mg while 8mg of melatonin was used in our study. Khezri et al did their postoperative haemoglobin after 12 hours while ours was done 48 hours post-operative.

The mean blood loss in melatonin group was 955.00ml, while the mean blood loss in placebo group was 1031.67ml. The difference between the two was not statistically significant with p-value of 0.097. This might be due to dosage of melatonin used. This finding was similar to that found by Khezri et al[13] and Jayeshree et al[53]. This similarity might be due to low dosage of melatonin, Khezri and Jayeshree et al used 3mg and 6mg of melatonin respectively and we used 8mg. The difference between 6mg and 8mg is not much. May be a higher dose of melatonin is needed. However, Khezri et al had found a significant blood in 6mg of melatonin that may not be

clinically meaningful[13] (Khezri et al did study with 3mg and 6mg of melatonin). This difference may be due to difference in sample size. Khezri et al used 40 participants per group while we used 162 participants per group. In addition, Khezri et al did postpartum haemoglobin after 12 hours, haemodilution may have not taken place. Our findings also differ from Javadi et al[54]. Javadi et al found that melatonin can significantly reduce blood loss during vaginal delivery. The difference may be due to the difference in dose of melatonin Javadi et al used 12mg of melatonin while we used 8mg. Also Javadi et al did their study among women that underwent vaginal delivery while our participants underwent elective caesarean section.

Furthermore, this study also demonstrated that pre-operation sublingual melatonin reduced the need for additional uterotonics during caesarean section compared to placebo which was not statistically significant. The possible explanation for this finding could be the gradual effect of melatonin which may not be clinically apparent. This finding was similar to that by Khezri et al[13] where there was insignificant difference in the dose of oxytocin used between the groups. This similarity may be due to the dosage of melatonin used by Khezri (6mg) and the dosage of melatonin used in this study. The difference is not much, 6mg and 8mg respectively.
In addition, this study demonstrated that melatonin reduced incidence of primary postpartum haemorrhage. This finding is similar to that of Jayeshree et al[53]. The similarity might be due to similar dosage regimen, 6mg and 8mg. Also it might be due to the fact that our participants and theirs underwent caesarean section.

Also, this study showed that there was increase in blood transfusion among the participants who received placebo more than those that received melatonin. This might be due to the fact that, melatonin augments oxytocin activity and enhances myometrial cell contractions leading to reduce blood loss[12,16]. The second possible reason is that, melatonin prevents high blood pressure and excessive haemorrhage by reducing anxiety[13]. The previous studies did not record their blood transfusion. Javadi et al excluded those that had blood transfusion and additional uterotonics[54].

Only minor side effects such as nausea, vomiting and headache were observed among the participants in this study. The difference between the two groups was not statistically significant. This may be due to the fact that we did not use very high dose of melatonin. This demonstrated that melatonin is safe and have no adverse maternal outcome. These findings were similar to that of Jayashree et al[53], this may be due to similarity in the dosage of melatonin, Jayeshree used 6mg while we used 8mg. Both are not very high dose of melatonin. This findings differ from that found by Khezri et al[13]. Khezri et al recorded that, the incidence of headache in 6mg melatonin group was significantly higher in melatonin group than placebo group. This difference may be due to the difference in sample size. Khezri et al use 40 participants per group while we used 162 participants per group.

Moreover, this study also noted that, there was no difference in the neonatal APGAR scores in first and fifth minute between the two groups and there was no new-born intensive care unit admission and no perinatal death. This might be due to the fact that we did not use very high dosage of melatonin and only those booked for elective caesarean section were recruited. This finding is similar to that found by Khezri et al[13]. This might be because, we and Khezri used a similar dosage of melatonin (8mg, and 6mg respectively). Also participants that were booked for elective caesarean section were recruited in our study as well as by Khezri et al. In addition, Javadi et al[54] also recorded a similar finding. This further suggests that melatonin did not have any adverse maternal or neonatal outcome.

CONCLUSION

This study demonstrated that 8mg sublingual melatonin did not significantly reduce blood loss at elective caesarean section. However, more participants in the placebo group required more oxytocic's and blood transfusion.

Consent

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL Approval:

Ethical clearance was sought and obtained from the Health Research and Ethics committee of the Alex Ekwueme Federal University Teaching Hospital, Mile 4 Hospital Abakaliki and National Obstetrics Fistula Centre Abakaliki.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCE

1. Mamah JE, Asiegbu OG, Asiegbu UV, Ekwedigwe KC, Nnadozie UU, OKafor L. A six-year review of caesarean sections at the Federal Teaching Hospital Abakaliki, Ebonyi State, South East Nigeria. Open J Obstet Gynaecol, 2020; 10: 1669-1676.
2. Bamigbala OA, Ojetunde AO, Ibrahim A. Assessing prevalence and factors associated with caesarean delivery among women of reproductive age in Nigeria. FJS 2022; 6 (1): 160-167.
3. Islam A, Sathi NJ, Hossain T, Jabbar A, Renzaho AMN, Islam MS. Caesarean delivery and its association with educational attainment, wealth index, and place of residence in sub-saharan Africa: a meta-analysis. Scientific report 2022; https://doi.org/10.1038/s41598-022-09567-1
4. Chaudhuri P, Majumdar A. Sublingual misoprostol as adjunct to oxytocin during caesarean section in women at risk of postpartum haemorrhage. Int J Gynaecol Obstet 2015; 125(1): 48-50.
5. Khan FA, Khan M, Ali A, Chohan U. Estimation of blood loss during caesarean section: an audit. J Pak Med Assoc, 2006; 56(12): 572-575.
6. Sotunsa JO, Adeniyi AA, Imaralu JO, Fawole B, Adegbola O, Aimakhu CO, A et al. Maternal near-miss and death among women with postpartum haemorrhage: a secondary analysis of the Nigeria Near-miss and Maternal Death Survey. BJOG, 2019; 126 (83): 19-25.
7. Garba Z, Abdullahi HM, Yusuf M, Takai IU, Muhammad ID. Appropriate documentation of the timing of events in the management of women with postpartum hemorrhage in Aminu Kano Teaching Hospital: A 2-year audit. Niger Med J 2019; 60: 9-12.s
8. Kalu FA, Chuhwurah JN. Midwives' experience of reducing maternal morbidity and mortality from postpartum haemorrhage (PPH) in Eastern Nigeria BMC Pregnancy Childbirth 22, 2022; 22 (474). DOI https://doi.org/10.1186/s12884-022-04804-x
9. Ezegwui HU, Onoh RC, Ikeako LC, Onyebuchi A, Umeora OUJ, Ezeonu P, Ibekwe P. Investigating Maternal Mortality in a Public Teaching Hospital, Abakaliki, Ebonyi State, Nigeria. Ann Med Health Sci Res 2013; 3: 75-80.
10. Green KI, Ojule JD, Faith MC. Primary postpartum haemorrhage at teaching hospital: Prevalence and risk factors. TNHJ, 2015; 15 (3): 111-117.
11. Obi VO, Umeora OUJ, Dimejesi IBO, Asiegbu O, Mgbafulu CC, Ifemelumma CC, et al. Efficacy of intravenous tranexamic acid at reducing blood loss during elective caesarean section in Abakaliki: A double blind randomized placebo-controlled trial. Afr J Med Health Sci, 2019; 18 (2): 10-17.

12. Sharkey JT, Cable C, Olcese J. Melatonin sensitizes human myometrial cells to oxytocin in a protein kinase C[α]/extracellular-signal regulated kinase-dependent manner. J Clin Endocrinol Metab. 2010; 95(6):2902–2908.
13. Khezri MB, Reihamy MD, Ghaleh TD, Mohammadu N. Effect of melatonin on blood loss after caesarean section: A prospective randomised double-blind trial. J Obstet Gynecol India 2019; 69: 436-443.
14. Pakniat H, Khezri MB. The effect of combined oxytocin–misoprostol versus oxytocin and misoprostol alone in reducing blood loss at caesarean delivery: a prospective randomized double-blind study. J Obstet Gynecol India. 2015; 65(6):376–381.
15. Tenorio FD, Simões MDJ, Teixeira VW, Teixeira AA. Effects of melatonin and prolactin in reproduction: review of literature. Rev Assoc Med Bras. 2015; 61(3):269–274.
16. Sharkey JT, Puttaramu R, Word RA. Melatonin synergizes with oxytocin to enhance contractility of human myometrial smooth muscle cells. J Clin Endocrinol Metab. 2009; 94(2):421–427.
17. Olcese J, Beesley S. Clinical significance of melatonin receptors in the human myometrium. Fertil Steril. 2014; 102(2):329–335.
18. Besag FM, Vasey MJ, Lao KS, Wong IC. Adverse events associated with melatonin for the treatment of primary or secondary sleep disorder: A systematic review. CNS Drugs. 2019; 33 (12): 1167-1186.
19. Sandall J, Tribe RM, Avery L, Mola G, Visser GH, Homer CS et al. Short-term and long-term effects of caesarean section on the health of women and children. Lancet 2018; 392: 1349–1357.
20. Nnadi DC, Singh S, Ahmed Y, Siddique S, Bilal S, et al. Maternal and foetal outcomes following caesarean deliveries: A cross-sectional study in a tertiary health institution in North-Western Nigeria. Sahel Med J 2016; 19(4):175.
21. Bulndi LB, Seljul RM, Ogundeko OT, Pual BA, Godwin AO, Godwin BI et al, Knowledge and Management of Postpartum Haemorrhage Among Skilled Birth Attendants in Primary Health Centres of Jos North LGA, Plateau State, World J Pub Health, 2017; 2(4): 124-130.
22. Obuna JA, Okafor I, Anikwe CC, Ejikeme BN, Eze JN. Visual estimation of blood loss post-delivery: How accurate are we in a poor resource setting. Int Res J Med Biomed Sci 2020; 5 (3): 24-28.
23. Nwafor JI, Ibo CC, Obi CN, Onwe B, Ugoji DC, Onuchukwu VU. Tranexamic Acid versus Placebo for Prevention of Primary Postpartum Haemorrhage among High Risk Women Undergoing Caesarean Section in Abakaliki: A Randomized Controlled Trial. Open J Obstet Gynecol, 2019; 9: 914-922
24. Olowookere AE, Adekeye O, Oluwayemisi O, Irinonye O, Ogunfowokan A. The prevalence, management and outcome of primary postpartum haemorrhage in selected health care facilities in Nigeria. IJNM, 5(3): 2013; 28-34.
25. Poggi SB. Postpartum haemorrhage & the abnormal puerperium. In Decherney AH, Nathan L, Lauren N, Laufer N, Roman AS. Current Diagnosis & Treatment Obstetrics and gynaecology. McGraw Hill 2019; 350.
26. Sujata N, Tobin R, Kaur R, Aneja A, Khanna M, Hanjoora VM. Randomized controlled trial of tranexamic acid among parturients at increased risk for postpartum hemorrhage undergoing cesarean delivery. Int J GynaecolObstet 2016; 133(3): 312-315.

27. Levy JH, Dutton RP, Hemphil JC. Hemostasis summit participants: multi-disciplinary approach to the challenge of hemostasis. AnesthAnlag 2010; 110: 354-364.
28. Wang F, Lu N, Weng X, Tian Y, Sun S, Li B. Measurement of postpartum blood loss using a new two-set liquid collection bag for vaginal delivery: A prospective, randomized, case control study. Med Baltimore, 2021; 100(19):e25906. doi:10.1097/MD.0000000000025906
29. Diaz V, Abalos E, Carroli G. Methods for blood loss estimation after vaginal birth. Cochrane Database Syst Rev. 2018; 9(9):CD010980. doi: 10.1002/14651858.CD010980.pub2. PMID: 30211952; PMCID: PMC6513177.
30. Kodkany BS, Derman RJ, Sloan NL. Pitfalls in Assessing Blood Loss and Decision to Transfer. In B-Lynch C, Keith LG, Lalonde AB, Karoshi M (eds) Textbook of Postpartum Haemorrhage: A comprehensive guide to evaluation, management and surgical intervention. Sapiens Publishing 2010; 81-88
31. Jayaraman S, Chalabi Z, Perel P, Guerriero C, Roberts I. The risk of transfusion transmitted infections in sub-Saharan Africa. Transfusion 2010; 50(2):433–42.
32. Nickson C. Major Haemorrhage in trauma. LITFL 2020; https://litfl.com/major.haemorrhage-in-trauma/
33. ACOG. Teaching module on postpartum haemorrhage. 2010.www.acog.org/ACOG_Districts/dist1jf/teachingmodulepostpartumhemorrhage.ppt-2010-01-20
34. Bose P, Regan F, Paterson BS. Improving the accuracy of estimated blood loss at obstetric haemorrhage using clinical reconstruction. BJOG 2006; 113(8):919-924.
35. Gerdessen L, Meybohm P, Choorapoikayil S, Herrmann E, Taeuber I, Neef V, et al. Comparison of common perioperative blood loss estimation techniques: a systematic review and meta-analysis. J Clin Monit Comput 202135**:** 245–258.
36. Smith CM, Border AE, King TL. Quantitative blood loss in obstetric hemorrhage. ACOG Committee Opinion No. 794. ACOG Obstet Gynecol 2019; 134: 150–6.
37. Al Kadri HM, Al Anazi BK, Tamim HM. Visual estimation versus gravimetric measurement of postpartum blood loss: a prospective cohort study. Arch Gynecol Obstet. 2011; 283(6): 1207-1213.
38. Ambardekar S, Shochet T, Bracken H, Coyaji K, Winikoff B. Calibrated delivery drape versus indirect gravimetric technique for the measurement of blood loss after delivery: a randomized trial.et al. Calibrated delivery drape versus indirect gravimetric technique for the measurement of blood loss after delivery: a randomized trial. BMC Pregnancy Childbirth, 2014;14**:** 276.
39. Wangwe PJ, Balandya B. Accuracy in diagnosis of postpartum haemorrhage using visual estimation of blood loss versus change in haematocrit in a tertiary teaching hospital in Tanzania. Tanzan J Health Res. 2012; 14(2):152-157.
40. Sneha K, Girish-Babu M, Manjunath ML. Haematocrit as diagnostic tool for assessing Primary Postpartum haemorrhage. Indian J Clin Anat Physio, 2018; 5 (2): 210-213.
41. Shields LE, Goffman D, Caughey AB. Postpartum haemorrhage. Practice Bulletin No 183. ACOG Obstet Gynecol 2017; 130 (4): 168-186.
42. Suseela TL, Jyothi SJ. Randomised controlled trial of tranexemic acid among parturient at increased risk of primary postpartum haemorrhage undergoing caesarean section in a tertiary teaching hospital at Rim Kadapa. Int J of app research 2016; 6(10): 75-50.

43. Kavle JA, Khalfan SS, Stoltzfus RJ, Witter F, Tielsch JM, Caulfield LE. Measurement of blood loss at childbirth and postpartum. Int J Gynecol Obstet 2006; 95 (1): 24-28.
44. Barret KE, Barman SM, Biotano S, Brooks HL. Electrical activity of the brain, sleep-wave states, and circadian rhythms. In Barret KE, Barman SM, Biotano S, Brooks HL, Ganong's Review of medical physiology 24th edition. McGraw Hill 2014; 278-280.
45. Katzung BG. Histamine, Serotonin and the Ergot Alkaloids. In Katzung BG, Masters SB, Trevor AJ (ed). Basic & clinical pharmacology 12th edition. McGraw Hill 2014; 281-283.
46. Emet M, Ozcan H, Ozel L, Yayla M, Halici Z, Hacimuftuoglu A. A Review of Melatonin, Its Receptors and Drugs. Eurasian J Med. 2016 Jun; 48(2): 135–141
47. Thorung P, Anuchita M, Loypima P. influence of pH, temperature and light on the stability of melatonin in aqueous solution and fruit juice. HELIYON 2020; 6 (3): 03649.
48. Khullar A. The Role of Melatonin in the Circadian Rhythm Sleep-Wake Cycle. Psychiatric Times 2020; 29 (7).
49. Ferlazzo N, Andolina G, Cannata A, Costanzo MG, Rizzo V, Currò M, et al . Is Melatonin the Cornucopia of the 21st Century? ANTIOXIDANTS. 2020; 9(11):1088.
50. Harpsøe NG, Andersen LP, Gögenur I, Rosenberg J. Clinical pharmacokinetics of melatonin: a systematic review. Eur J Clin Pharmacol 2015; 71 (8): 901-909.
51. Wurtman R, Cooper DS, Martin KA. Physiology and available preparation of melatonin. Uptodate 2021; [cited 2021 July 20] available from: https://www.uptodate.com/contents/physiology-and-available-preparations-of-melatonin?csi=9725d18c-5e7e-4296-bcf0-ddb4e273a904
52. Schlabritz N, Heliner N, Midderndorf R, Muller D. The human myometrium as a target for melatonin. J Clin Endo Metab, 2003; 88 (2): 908-913.
53. Jayashree V, Vanathi R, Baby M. Effect of melatonin in reducing blood loss during and after caesarean section. Inter J Clin Obstet Gynae 2021; 5 (6) 227-231.
54. Javadi EH, Kamali M, Brikani, Lalooha F, Khezri MB. Effect of melatonin on postpartum haemorrhage in vaginal delivery: a prospective randomized double-blind study. Italian J Gynae Obstet 2021; 33 (3): 197-202.
55. Vine T, Brown GM, Frey BN. Melatonin use during pregnancy and lactation: A Scooping review of human studies. Braz J Psychiatry. 2021; 44 (3): 342-348.
56. Voiculescu SE, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo foetal development. J Med Life. 2014; 7(4): 488-492.
57. Unifer V, Raffone E, Rizzo P, Buffo S. Effect of supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecol Endocrinol. 2011; 27 (11): 857-861
58. Chuku EO. Estimate of rural poverty level and income distribution in Ebonyi state of Nigeria. Nig J Agric, Food Environ 2012; 8 (3): 52-61.
59. 2013 demographic and health survey (NDHS); south east. [Cited 2021 August 13]. Available from; https://dhsprogram.com>pubs>pdf.
60. Wikipedia. Ebonyi state. [Cited 2021 August 13]. Available from; https:en.m.wikipedia.org/wiki/Ebonyi_state.
61. Lawani OL, Iyoke CA, Onyebuchi AK. Blood transfusion trends in Obstetrics at Federal Teaching Hospital Abakaliki, South-East Nigeria. Int J women Health 2013; 5: 407-412.
62. Zhong B. How to calculate sample size in Randomized Control Trial. J Thorac Dis 2009; 1(1): 51-54.