

Role of Endometrial Scratch in UnExplained infertility (RESCUE); A Randomized Clinical Trial

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Abstract

Background

Endometrial natural killer (NK) is thought to play a role in implantation and maintenance of a healthy pregnancy. However, their immunological role in unexplained infertility is yet unknown. The aim of the current study was to investigate the role of endometrial injury in treatment of unexplained infertility and to have more insight on the potential underlying mechanisms of its action methodology randomized controlled trial (RCT) done at Minia university hospital, diagnostic laparoscopy was done for both study and control group, study group had gentle scratch and specimens were sent for histopathological examination while control group had Laparoscopy only .Patients in both groups were followed up at the outpatient clinic for 3 months to assess if pregnancy achieved or not. Patients in the study group who had not achieved pregnancy had a repeated scratch using a pipelle sampler which was also sent for histopathology. Patients were counselled for intrauterine insemination (IUI) with ovulation induction while patient in control group had been counselled for IUI and ovulation induction only. Patients in both groups were followed up for further 3 months to assess patients who achieved pregnancy either naturally or after IUI .

Results

Baseline characteristics were comparable in both groups. Clinical pregnancy rate (CPR) was significantly higher in scratch group compared with the control group (22%; vs. 7.4%, $P = 0.001$) in the first three months of follow up. After second scratch and IUI cycles cumulative pregnancy rate was significantly higher in the scratch group compared with the control group (36.7% , vs. 16.2%, $P = 0.001$). The mean time achieve pregnancy was found to be significantly shorter in the scratch group as compared to the control group (102 days vs.49.5 days, $P = 0.01$).

Conclusion:

Endometrial natural killer cells might play a crucial role in endometrial receptivity and therefore embryo implantation. Endometrial scratch could promote the recruitment of endometrial NK cells and give a more favourable results as regard clinical pregnancy in patients with unexplained infertility.

Registration number PACTR201604001405465

Introduction

Endometrial curettage (injury) has been shown to improve the pregnancy and live birth rate following recurrent implantation failure with 3 systematic reviews showing consistently improved pregnancy and live birth rate when endometrial injury (EI) or scratch was done in the luteal phase preceding IVF treatment.(Ref) The exact mechanism of action of EI is however not clearly understood. Proposed mechanism of action includes promoting recruitment of a more favourable repertoire of immunological

cells in the endometrium with a profile of growth factors and inflammatory cytokines conducive of implantation. Although the role of endometrial injury has been investigated in ART population its role as a primary tool for infertility treatment outside ART treatment is not clearly established. In patients with unexplained infertility it is possible that one of the underlying mechanisms is a defective implantation process which could be corrected by endometrial injury

Endometrial scratching was favorable practice has been widely implemented in daily procedure to diagnose or treat infertility (1)(2)(3)(4). It is defined as an intentional damage caused to the endometrium (5). The connection between local injury to endometrium and increased pregnancy rates in IVF cycles was first described in 2003 (6).

However it is still unclear if endometrial scratching improves the chance of a live birth and, if so, which population benefits most (7)(8)(9) (10)(11)

Natural killer (NK) cells could be considered the single most important determinants of the success of human reproduction. While measuring them and attempting to manipulate their numbers and function are causes of much controversy,(12)

A recent systematic review of available literature suggests that the prognostic value of pNK or uNK cell testing is uncertain when predicting IVF treatment outcome. The lack of appropriately powered studies is due to varying inclusion criteria, different NK cell testing methodology and varying outcome measures (13).

In this study we aim to investigate the role of endometrial injury in treatment of unexplained infertility and to have more insight on the potential underlying mechanisms of its action.

Patients And Methods

This clinical trial research was done in Maternity Hospital Minia University during the period from March 2016 till April 2017.

Patients who are undergoing infertility investigation in Minia university hospital were recruited for the study if they fulfil the following eligibility criteria

- Female age is < 35 years
- Duration of subfertility <3 years
- Unexplained infertility (confirmed with semen analysis, basic hormonal profile, pelvic ultrasound and diagnostic laparoscopy and dye test)
- No history of previous ART

Patients who fulfil the previous criteria were enrolled for the study after obtaining written informed consent and were booked for diagnostic laparoscopy as part of their infertility work-up to coincide with their luteal phase preferably between day 17-24 of their cycles after performing a sensitive urine pregnancy test on the day of the laparoscopy to exclude luteal phase pregnancy. At laparoscopy after confirming unexplained infertility, patients were randomised to two groups

Group A

had gentle endometrial curettage (endometrial injury) by the use of sharp curette on the anterior and posterior wall for 1 minute and specimen was sent for histopathological investigation.

Group B

Laparoscopy procedure was continued as per routine procedure without additional endometrial curettage.

Patients in both groups were followed up in the outpatient clinic for 3 months and were contacted if not attending clinic (contact details of patients were taken in advance to avoid loss of follow-up) to assess if they have got pregnancy or not.

At the first follow-up visit to assess patients in group A who did not get pregnant were have a repeat endometrial scratch using a pipelle endometrial sample in the clinic which was also sent for histopathology. Patients were counselled for intrauterine insemination with ovulation induction whilst patient in group B have counselling for IUI and ovulation induction without any further intervention. Both groups were followed up for further 3 months to assess patients who achieved pregnancy whether naturally or after IUI

Specimens sent for histopathology were assessed by immunohistochemistry for the population of uterine natural killer cells (uNK) by the use of CD56 marker as well as by routine H&E section to assess for decidual reaction.

Histopathological examination:

Specimens sent for histopathology were assessed by a pathologist who was blind to the different random groups but informed by the study design. The specimens were fixed for 24h in 10% formaldehyde, processed and paraffin embedded. Multiple 5µm sections were cut from each case and stained by hematoxylin and eosin stains. Examination by light Olympus microscope CX41 (Olympus, Tokyo, Japan) to assess the efficiency and diagnosis of the cases as well as to assess for decidual reaction. Photos were taken for selected sections were taken using an Olympus microscopic camera (C5050Z, Olympus, JP).

Immunohistochemistry:

Multiple 5µm sections were cut from all studied cases for immunohistochemical analysis of the uterine natural killer cells population (uNK) using CD56 marker. Sections were all subjected to xylene for

deparaffinization followed by descending grades of alcohol for rehydration. Blocking of endogenous peroxidase activity was done using 3% H₂O₂ and washing in phosphate buffer solution (PBS). Antigen unmasking was done for all slides by citrate buffer pH 6.0 in microwave for 30min. After cooling down to the room temperature, the slides were incubated with the primary antibody cd56 (Santa Cruz Biotechnology, ready-to-use) overnight in a humidity chamber at 4°C. Secondary biotinylated antibody (Lab Vision) was applied after rinsing with PBS for 30min. Incubation for 30min was applied with streptavidin-biotin (Lab Vision) followed by DAB treatment of the slides for 5 min to obtain the brown color of the positive cells. Finally, the slides were washed with water and counter stained with Mayer's hematoxylin. Dehydration, clearing, mounting of the slides was done followed by covering with slips. Positive and negative control slides were processed with the cases in each run.

Immunohistochemical scoring:

Sections were examined under Olympus light microscope to count the number of positive cells in at least nine 400x high power fields and at three different sites per slide if sufficient area was available (14)

Sample calculation:

The number required for recruitment for the study was 136 (68 patients in each arm) patients assuming a difference in cumulative pregnancy rate between the two arms of 20% and for type 1 error of 0.05 and power of 80% and assuming a drop-out rate of 10%

The outcome measures:

Primary cumulative pregnancy rate over 6 months of follow-up

Secondary: Time to pregnancy (in days) , complication secondary to scratch.(bleeding ,infection)

The trial was registered prospectively in Pan African clinical site Registry with unique registration number PACTR201604001405465

Results

A total of 136 patients presenting with primary or secondary infertility (unexplained infertility) were screened and assessed for eligibility criteria. Of these, patients were randomized into intervention group ($n = 68$) and control group ($n = 68$)

Table 1 and 2 demographic characters

		Control group (n = 68)	Scratch group (n = 68)	P value
Age	<i>Range</i>	(19-35)	(19-35)	0.442
	<i>Mean ± SD</i>	26.6±4.1	26.1±3.6	
BMI	<i>Range</i>	(18-34)	(18-33)	<0.131
	<i>Mean ± SD</i>	24.3±3.6	23.7±3	
Infertility type	<i>1ry</i>	54(79.4%)	60(88.2%)	0.162
	<i>2ry</i>	14(20.6%)	8(11.8%)	
Infertility duration	<i>Range</i>	(1-3)	(1.2-3)	0.666
	<i>Mean ± SD</i>	2.1±0.7	2.2±0.6	

Miscarriage	<i>Mean± SD</i>	0.7± 0.25	0.71± 0.45	0.338
Parity	<i>Mean± SD</i>	0.64±0.24	0.73± 0.34	0.099
AFC	<i>Range</i>	(9-18)	(9-18)	0.859
	<i>Mean ± SD</i>	13.9±2.6	13.8±2.2	
AMH	<i>Range</i>	(1.9-4.6)	(1.9-4.9)	0.732
	<i>Mean ± SD</i>	3.3±0.8	3.3±0.7	

Table 23 and 4 show primary and secondary outcome

	Number and percentage
Pregnancy rate (total) (N=126)	90(71.4%)
	36(28.6%)
Pregnancy rate after 1 st scratch (N=68)	15(22.1%)
Pregnancy rate after 2 nd scratch (N=53)	10(18.8%)
Pregnancy rate in control cases(N=68)	11(16.2%)

	Control	Cases	P value
Outcome	11(16.2%)	25(36.7%)	0.001*
Days till pregnancy	102 (66-143)	49.5 (33.5-120.8)	0.01*

Table 5 Demographic features in the pregnant and non-pregnant populations.

		Not Pregnant N=90	Pregnant N=36	P value
Age	<i>Range</i>	(19-35)	(19-35)	0.582
	<i>Mean ± SD</i>	26.6±3.9	26.2±3.8	
BMI	<i>Range</i>	(18-35)	(19-34)	0.663
	<i>Mean ± SD</i>	25.2±3.6	24.9±3.6	
Infertility type	<i>1ry</i>	75(83.3%)	29(80.6%)	0.771
	<i>2ry</i>	15(16.7%)	7(19.4%)	
Infertility duration	<i>Range</i>	(1-3)	(1-3)	0.028*
	<i>Mean ± SD</i>	2.2±0.6	2±0.6	
Abortion	<i>Median</i>	0	0	0.185
	<i>IQR</i>	(0-0)	(0-0)	
Parity	<i>Median</i>	0	0	0.504
	<i>IQR</i>	(0-0)	(0-0)	
AFC	<i>Range</i>	(9-18)	(9-18)	0.783
	<i>Mean ± SD</i>	13.9±2.4	13.7±2.5	
AMH	<i>Range</i>	(1.9-4.6)	(2.1-4.9)	0.470
	<i>Mean ± SD</i>	3.3±0.8	3.2±0.8	

Table 6 compare CD 56 scoring in pregnant and non-pregnant after first and second scratch

				P value
		Not Pregnant	Pregnant	
CD 56 (1st)	<i>Median</i>	0	2	<0.001*
	<i>IQR</i>	(0-1)	(2-3)	
CD 56 (2nd)	<i>Median</i>	1	2	<0.001*
	<i>IQR</i>	(0.5-2)	(2-3)	
<i>P value (1st vs 2nd)</i>		0.001*	0.166	

Table 7 (histopathological diagnosis)

		N=68
Diagnosis	Proliferative endometrium	
	<i>Disordered prolif.</i>	7(10.3%)
	<i>Proliferative with scanty secretory changes</i>	13(19.1%)
	<i>Disordered proliferative. With hormonal effects</i>	5(7.4%)
	<i>Simple endometrial hyperplasia no atypia</i>	9(13.2%)
	<i>Simple endometrial hyperplasia with atypia</i>	7(10.3%)
	<i>Complex hyperplasia without atypia</i>	3(4.4%)

Table 8

		N=68
Side effects	No	
	<i>Spotting</i>	3(4.4%)
	<i>Postoperative pain</i>	1(1.5%)

Discussion

uNK cells are the numerous types of leukocytes in the human endometrium during the window of implantation and considered to play a fundamental role in implantation and maintenance of pregnancy. It supposed to release a unique pattern of immune and angiogenic factors during their interactions with the trophoblast during the early gestation. natural killer (NK) cells in the uterus promote successful pregnancies by regulating the depth of placental trophoblast invasion. (15)

The present study was conducted to evaluate the role of endometrial scratching and uterine NK cells in cases with unexplained infertility. Our results showed significantly higher clinical pregnancy rate in patients undergoing endometrial scratching than control cases that undergo same management without scratch 43.1% versus 17.7% respectively with significant (P value 0.001).

Endometrial NK cells have little cytotoxic activity, but are a rich source of cytokines, particularly angiogenic ones, which are possibly involved in tissue remodelling and angiogenesis. a reliable method to determine which women might benefit from manipulation of the maternal immune system are not yet done and is urgently needed(16)

In cases had endometrial scratch the endometrial CD56+ NK cells are stimulated for differentiation in the endometrium. and involved in the regulation of trophoblastic invasion that explain the high pregnancy rate in the study group .The role of endometrial CD56+ NK cells in the immunological mechanism of unexplained infertility is still under research. Quantification of endometrial CD56+ NK cells, in the proliferative phase and its correlation to occurrence of pregnancy in cases of unexplained infertility was first done in our research. We noticed that the median scoring of CD56 NK cells increased in study group who had pregnancy(median CD 56 scoring 2) than study group hadn't (median CD 56 scoring zero) with significant P value(<0.001)

Our study shows strong positive association between CD56 scoring and occurrence of clinical pregnancy this supported by Yang et al who reported successful implantation requires a fine-tuned and highly regulated balance between the maternal immune system and the fetus .The maternal-fetal interface consists of different immune cells, such as (NK) cells, macrophages, T cells, dendritic cells, B cells.. Dynamic changes in immune cells at the maternal-fetal interface have not been clearly stated.(17)

atching was suggested by Gnainsky et al. [18]. The authors observed that endometrial biopsy induced an inflammatory response that perhaps facilitates the preparation of the endometrium for implantation.

The pregnancy rate did not improved significantly with repeated scratch this was in consistence with findings reported by Gnainsky et al. [18]who suggested that the effect of endometrial scratching is long lived as monocytes recruited to the injured sites are long lived and reside in tissues for a long time. Such beneficial effect even up to 6 months has been reported by Gibreel et al. [19] who reported increased cumulative pregnancy rates for 6 months.

One RCT has shown an impressive improvement in cumulative pregnancy rate of 25.9% within 6 months of endometrial injury as compared with 9.8% within 6 months without endometrial injury in cases of

unexplained infertility this is consistent with results of our study as cumulative pregnancy rate in study group was ..versus ..in the control group. the total number of uterine CD56⁺ cells seems to play a relevant role in increase rate of pregnancy. the data reported above suggests CD56⁺uNK cells, may be more important for maintaining a supportive environment for the establishment of pregnancy. However, further investigation on larger populations of women is needed to confirm this result.(Giuliani et al 2014). This finding has not been corroborated by other RCTS. Contrary to previous data, did not find any benefit from local injury to the endometrium this may attributed to difrrent inclusion citreia and different study procedure they concluded that Further studies are warranted to better define the target population of patients who may benefit from this procedure.(20)(21)

Abbreviations

NK natural killer

randomized controlled trial (RCT)

IUI intrauterine insemination

uNK uterine natural killer

Declarations

- Ethics approval and consent to participate the study was approved by the institutional ethical committee of Faculty of medicine, Minia University (Registration number: MUH16730). All patients were invited for involvement in this research and they gave an informed consent for their approval.
- Consent for publication: not applicable
- Availability of data and materials The datasets analyzed during the current study are available from the corresponding author on reasonable request
- Competing interests, the authors declare that they have no competing interest.
- Funding none
- Authors' contributions All the authors have made significant contribution to the manuscript. RE, AM, HB, and MM were involved in the conception and design of the study. All authors participated in the initial data collection, planning of the analysis and interpretation of results. AG, AE, and MI performed the literature review and drafted the manuscript. RE,AM,HB,MI prepared the final version of the manuscript. All authors have read and approved the final version of the manuscript.Nisreen Toni was responsible for the practical part of pathology and immunohistochemistry.

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Figures

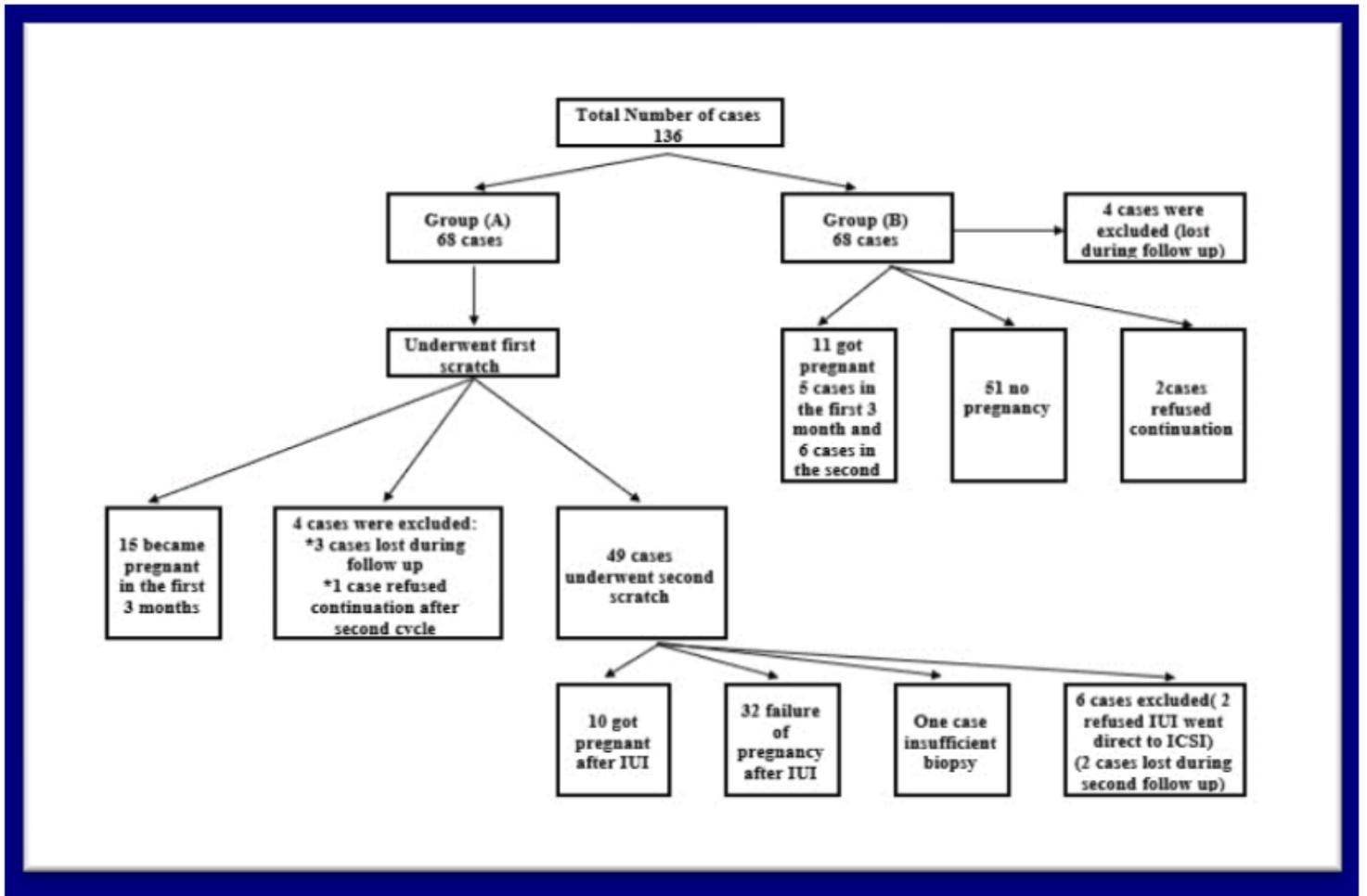


Figure 1

flowchart of study and control group: