

## Original Research Article

## EXPRESSION OF C-KIT IN INTERSTITIAL CELLS OF CAJAL OF FALLOPIAN TUBE OF TUBAL ECTOPIC GESTATION- 3 YEAR STUDY

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Received : 17/01/2023  
 Received in revised form : 25/02/2023  
 Accepted : 09/03/2023

**Keywords:**  
 Tubal ectopic gestation, interstitial cells of cajal, pelvic inflammatory disease.

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DOI: 10.47009/jamp.2023.5.2.193

Source of Support: Nil,  
 Conflict of Interest: None declared

Int J Acad Med Pharm  
 2023; 5(2); 912-916



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**Abstract**

**Background:** Ectopic pregnancy is one of the major cause of maternal death in early pregnancy. Fallopian tube is involved in transport of zygote to the uterine cavity by peristaltic action of muscles of the fallopian tube and by the synchronous beating of cilia. **Materials and Methods:** A 3-year study conducted from September 2014 to August 2017, in department of pathology, Modern Government maternity hospital, Petlaburz Hyderabad. **Result:** A total of 110 cases of fallopian tubes with tubal ectopic pregnancy and 40 normal fallopian tubes were taken for this study. We found significant reduction in number of interstitial cells of cajal (ICC) compared to normal fallopian tubes with statistically significant P value<0.0001. **Conclusion:** Absence or decreased number of interstitial cells of cajal (ICC) in the fallopian tube is main reason of tubal ectopic pregnancy which is caused by major risk factors like infections (Chlamydia etc) and tubal surgeries.

**INTRODUCTION**

Ectopic pregnancy is one of the major cause of maternal death in early pregnancy.<sup>[1]</sup> Ectopic pregnancy occur in approximately 1.5-2% of pregnancies and result in 10% of all pregnancy related deaths.<sup>[2]</sup> This condition currently is the leading cause of pregnancy related deaths during the first trimester. Tubal ectopic pregnancies are the most common, representing more than 97% of ectopic pregnancies.<sup>[3]</sup> Fallopian tube is involved in transport of zygote to the uterine cavity by peristaltic action of muscles of the fallopian tube and by the synchronous beating of cilia.<sup>[4]</sup> In addition to the gastrointestinal tract, Interstitial cells of cajal (ICC) have been found in the mammary gland, myocardium, urinary tract, testes and fallopian tube.<sup>[5]</sup> Interstitial cells of cajal (ICC) generate electrical activity in the form of slow waves generating rhythmical contractions of the smooth muscle.<sup>[6]</sup> Gastrointestinal tract motility disorders occur due to absence or deficiency of Interstitial cells of cajal (ICC), similarly human fallopian tube may also have an effect on motility due to decreased number of Interstitial cells of cajal (ICC) and retention of the embryo within the fallopian tube, resulting in an ectopic pregnancy. Our study shows the number and distribution of interstitial cells of Cajal (ICCs) in fallopian tubes with ectopic gestation in comparison to normal fallopian tubes by immunohistochemistry using anti c-kit antibody.

**Objective**

To study the number and distribution of interstitial cells of Cajal (ICCs) in fallopian tubes with ectopic gestation in comparison to normal fallopian tubes by immunohistochemistry using anti c-kit antibody.

**MATERIALS AND METHODS**

The present study is a 3-year study conducted from September 2014 to August 2017, in Upgraded department of pathology, Modern Government maternity hospital, Petlaburz Hyderabad. A total of 110 cases of fallopian tubes with tubal ectopic pregnancy and 40 normal fallopian tubes were taken for this study.

Specimens are immediately fixed in 10% buffered formalin kept overnight and grossly examined with proper orientation of specimen. Representative sections were taken, then dehydrated in ascending grades of alcohol and cleared in xylene then embedded into paraffin wax. After embedding sections were obtained by microtomy and sections of 4u thickness were subjected to histological staining using hematoxylin and eosin.

Immunohistochemistry C-Kit (clone EP10) performed on 4 micron sections utilizing standard protocols on all selected cases.

**RESULTS**

In our study majority of patients with tubal ectopic gestation are in the age group 20 - 25 years (50%),

majority of them were primi gravida (44.54%), (63.63%), with right side being most common presented at gestational age of 6-7weeks (45.45%), (68.81%). most common symptom being pain abdomen

**Table 1: showing various parameters of our study.**

Parameter	
Most common age	20-25years
Parity	Primigravida
Gestational age	6-7 weeks
Common symptom	Pain abdomen
Common side	Right

Immunohistochemistry done with CD117(C-KIT) on 110fallopian tubes with ectopic gestation and in 40 normal fallopian tubes. Mean count and distribution of interstitial cells of Cajal (ICC) were done.

The distribution and number of Interstitial cells of Cajal (ICC) were assessed in the muscularis layer and in the lamina propria of 10 non-overlapping high-power fields, using a light microscope (Olympus CH series) at a magnification of x 400 (using X40 objective lens). Interstitial cells of Cajal have oval shaped nucleus and cytoplasmic extensions. As CD117 also highlights mast cells but morphologically mast cells are round without any cytoplasmic extensions. Also used toluidine blue stain which is a metachromatic stain to stain granules of mast cells and to differentiate mast cells from interstitial cells of Cajal.

**Table 2: Mean ICC counts per high power field (+ standard deviation) in the muscularis layer and lamina propria of normal fallopian tube.**

AGE	No. Of cases	MUSCULARIS MEAN + SD	LAMINA PROPRIA MEAN + SD
20-25 years	02	5.55 + 0.77	3.90 + 0.14
26-30 years	02	5.80 + 0.42	4.00 + 0.07
31- 35 years	10	5.37 + 0.46	3.56 + 0.40
>35 years	26	5.27 + 0.39	3.40 + 0.52

**Table 3: Mean ICC counts per high power field (+ SD) in the muscularis layer and lamina propria of ectopic gestation fallopian tube.**

AGE	No. of cases	Muscularis mean + SD	Lamina propria + SD
<20 years	02	3.20 + 0.141	1.2 + 0.141
20 – 25 years	55	3.15 + 0.142	1.38 + 0.277
26 – 30 years	36	3.16 + 0.131	1.45 + 0.257
31 – 35 years	16	3.15 + 0.109	1.34 + 0.230
>35 years	01	03	01

Mean count of interstitial cells of Cajal (ICC) in muscularis of normal fallopian tube (Mean+SD) is 5.33 + 0.42/hpf and in lamina propria of normal fallopian tube (Mean + SD) is 3.50 + 0.49/hpf. Mean count of interstitial cells of Cajal (ICC) in muscularis of tubal ectopic gestation is (Mean+ SD) 3.14 + 0.13/hpf and in lamina propria is 1.39 + 0.26/hpf.

**Table 4: Mean ICC Counts (± standard deviation) per high power field in the muscularis layer of normal FTS and FTS with ectopic gestation**

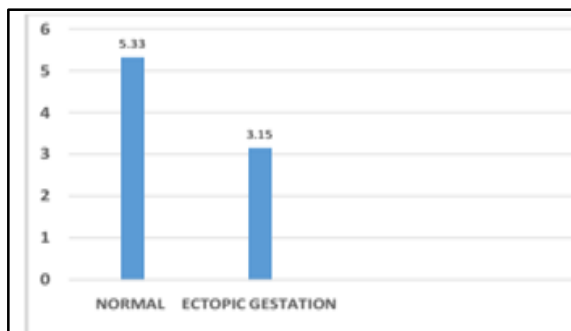
GROUP	No. of cases	Mean + SD	P value
Normal	40	5.33 + 0.428	<0.0001
Ectopic gestation	110	3.15 + 0.133	

p- value was obtained using Unpaired t- test

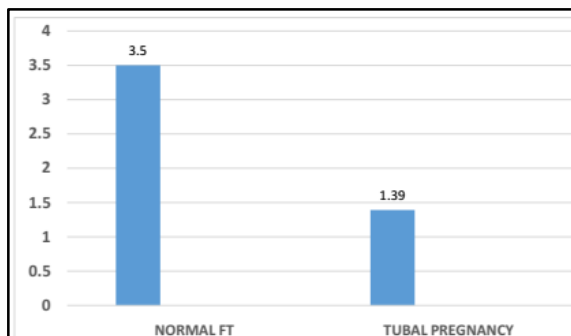
**Table 5: Mean ICC Counts (± Standard Deviation) Per High Power Field In The Lamina Propria Of Normal FTS and FTS with Ectopic Gestation.**

GROUP	No. Of cases	Mean + SD	P value
Normal	40	3.50 + 0.494	<0.0001
Ectopic gestation	110	1.39 + 0.266.	

p- value was obtained using Unpaired t- test



**Comparison of Mean Count of ICC in muscularis layer of Normal and Ectopic Gestation**

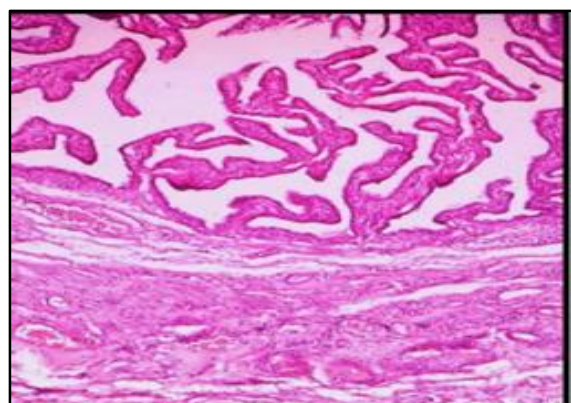


**Comparison of mean ICC count in lamina propria of normal fallopian tube and ectopic gestation fallopian tube**

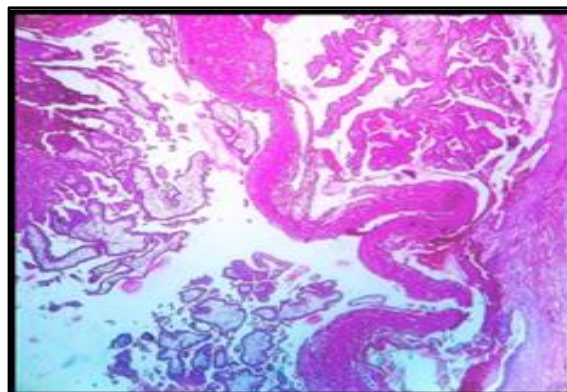
Mean count of interstitial cells of cajal when both groups are compared showed 41.5% reduction in muscularis and 60.2% reduction in lamina propria of ectopic gestation fallopian tube, with statistically significant p value of <0.0001.



**Figure 1: Gross photograph showing fallopian tube with fetal contents and hemorrhage.**



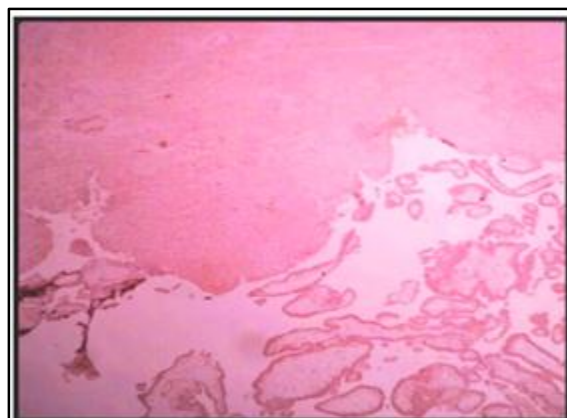
**Figure 2: H&E Normal fallopian tube 10X**



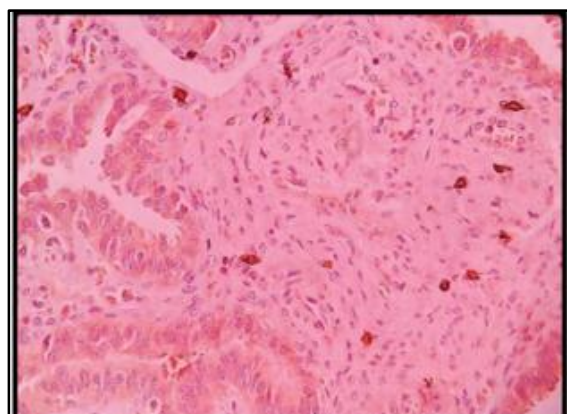
**Figure 3: H&E - section of tubal ectopic gestation.10X**



**Figure 4: CD117 positive cells in normal fallopian tube. 10X**

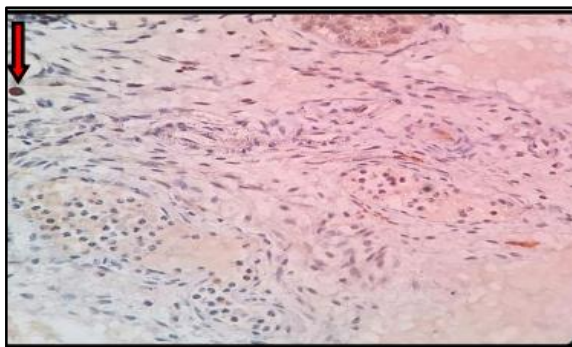


**Figure 5: CD117 positive cells in tubal ectopic gestation fallopian tube.10X**

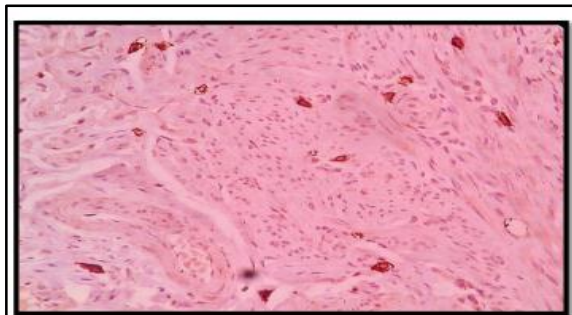


**Figure 6: CD117 positive ICC and mast cells in lamina propria of normal fallopian tube 40x**





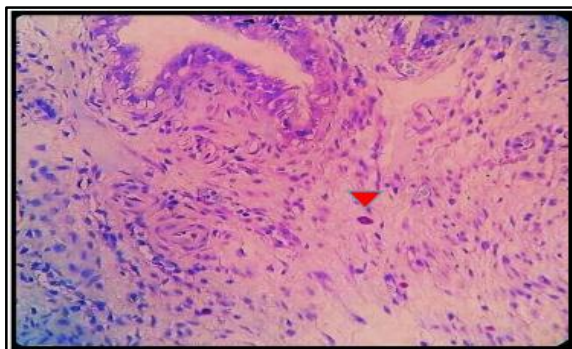
**Figure 7: CD117 positive ICC and mast cell (round) in lamina propria of tubal ectopic gestation.40X**



**Figure 8: CD117 positive ICC (spindle shaped) and mast cells in muscularis of normal fallopian tube.40X**



**Figure 9: ICC in muscularis of tubal ectopic gestation.40X**



**Figure 10: Toluidine blue stain showing mast cell (arrow head) with metachromatic granules.40X**

## DISCUSSION

Ectopic pregnancy is the leading cause of maternal mortality in the first trimester of gestation resulting from acute abdominal bleeding.<sup>[7]</sup> An ectopic pregnancy is of greater importance because of its increasing incidence and its impact on future fertility.<sup>[8]</sup>

In India the incidence of ectopic pregnancy reported by the Indian council of medical research (ICMR 1990) task force in their multicentric case control

study was 3.12 per 1000 pregnancies or 3.86 per 1000 live births in the hospital reported pregnancies.<sup>[9]</sup>

In the study by Shukla DB et.al (2017) in India, reported tubal ectopic gestation most commonly seen in the age group 21 - 25 years.<sup>[10]</sup> In the study by Jani RS et.al, (2014) in India, shows tubal ectopic gestation most commonly occurs in the age group of 21-25 years.<sup>[11]</sup> In our study most common age group showing ectopic pregnancy is 21-25 years, compared to older adults as this age group are at risk of acquiring sexually transmitted infections (STI) and developing Pelvic inflammatory diseases which is one of the major cause of ectopic pregnancy because of their behavioral, biological and cultural reasons.

In our study majority of the cases of tubal ectopic gestation were seen in primigravida (44.54%) followed by Gravida 2 in 25.45% and gravida 3 in 17.27% of cases. This is in concordance with study by Islam et al (2017) India where majority of the cases of are seen in primi gravida (31.3%), followed by 20.0% in Gravida2, 11.1% in Gravida 3.

Majority of patients in the present study showed most common site of tubal ectopic gestation as ampulla (59.09%), followed by isthmus (27.27%) this is in concordance with the study of Shukla DB et al (2017) where ampulla was the most common site (65.97%), followed by isthmus (27.83%).<sup>[10]</sup>

Majority of the cases in our study showed right sided tubal ectopic gestation (61.81%), followed by left side tube (38.18%) this is in concordance with various studies. Shukla et al (2017) reported 55% of cases had right side ectopic pregnancy and 45% on the left side.<sup>[10]</sup>

Compared to other studies by Shukla DB et.al (2017), Jani RS et.al, (2014), Islam et al (2017) there was a similarity observed in the present study in age group, gravida and common side of tubal ectopic gestation. In this study we also studied the role of Interstitial cells of cajal and its significance in tubal ectopic gestation. Interstitial cells of cajal are pace maker cells which are generally seen in gastrointestinal tract helps in peristaltic movements. There are other previous studies on interstitial cells of cajal in fallopian tubes and its function in peristalsis of fallopian tube which helps in movement of zygote from fallopian tube to uterus. To identify Interstitial cells of cajal IHC CD117 was used. In the present study toluidine blue stain was used to demonstrate interstitial cells of cajal (ICC) and their cell processes, which also helps in differentiating Interstitial cells of Cajal with mast cell as both the cells are CD 117 positive. With toluidine blue stained mast cells appeared as round and showed purple metachromatic granules in their cytoplasm.

In the present study two study groups are compared for distribution and number of Interstitial cells of cajal (ICC) and it showed significant decrease in interstitial cells of cajal (ICC) number in both lamina propria and muscularis of the fallopian tubes of tubal ectopic gestation. This is in concordance with studies by Ahmed et al (2006) and khoo et al (2014).<sup>[14,15]</sup>

Shafik et al (2005) postulated that these Interstitial cells of cajal discharge electric waves that effect tubal motility. They appear to act as pacemaker cells, which are probably responsible for the generation of the fallopian tube motile activity and transport of the zygote from the fallopian tube to the uterus.<sup>[16]</sup>

In the present study larger number of fallopian tubes with ectopic gestation comprising of 110 cases were studied, and found significant reduction in number of interstitial cells of cajal (ICC) compared to normal fallopian tubes with statistically significant P value<0.0001.

Ectopic pregnancy still remains a very serious threat to maternal safety even though there are advances in diagnostic methods and management. Identifying the risk factors and taking steps to reduce them, improves morbidity, mortality, and fertility outcomes.

There are multiple causes for the decreased number of interstitial cells of cajal in fallopian tube, one of the most important cause is pelvic inflammatory disease which causes fallopian mucosal damage and decreased number of Interstitial cells of cajal. In India, the incidence of genital tuberculosis in patients prone to acute ectopic pregnancy was is also high 35.29–40%. This may be due to tubal damage resulting in scarring and fibrosis of fallopian tube and also damage of interstitial cells of cajal causing tubal ectopic pregnancy.<sup>[17]</sup>

It has been stated that Interstitial cells of cajal exert pace making activity via voltage dependant channels, generate electrical activity in the form of slow waves. Smooth muscles express voltage dependant calcium channels which respond to slow waves generating rhythmical contractions of the smooth muscle.

There are large number of studies on gastrointestinal motility disorders which states decreased peristalsis due to decreased conduction of slow waves along the gastrointestinal tract due to defect in Interstitial cells of cajal.

In future further studies are needed to prevent ectopic gestation thereby decreasing maternal mortality in first trimester and improving fertility in women.

## CONCLUSION

Even with advancement of sophisticated diagnostic aids ectopic pregnancy incidence is on rise, and it is still a life threatening cause of maternal mortality. Understanding cause of tubal ectopic pregnancy is necessary for the prevention of tubal ectopic pregnancy. Majority of the cases may not have identifiable risk factor causing diagnostic problem. Absence or decreased number of interstitial cells of cajal (ICC) in the fallopian tube is main reason of tubal ectopic pregnancy which is caused by major risk factors like pelvic inflammatory diseases, genital tuberculosis and tubal surgeries.

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