Histological Changes in VAS Deferens after Chemical Vasectomy

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ABSTRACT

Introduction: Vasectomy as contraceptive is being used for so many decades. Aim and objective of present study was to find out the histological changes in vas deferens after injection of various chemical agents as sclerosing agents as a method of male sterilization.

Material and Methods: Present clinical study was carried out in L.L.R Hospital, Kanpur, during the period of August 1982 to June 1983. Forty five patients were classified in 3 equal groups by using three different chemical agents. Two to six weeks following injection, biopsy of vas deferens was taken and histological examination was done.

Results: Optimum amount of sclerosing chemical agent to bring histological obliteration was 0.2-0.3 ml. Chemical agents in vas for sclerosing has given quite satisfactory response with 95% ethanol showing most satisfactory changes in vas, desired for obliteration. Maximum histological changes occur latest by 4th week and then become stationary, since in biopsy at 6 weeks interval, in the same group, histological changes in vas were almost same as at 4 weeks.

Conclusion: Therefore it can be concluded that best results are achieved with 95% ethanol in comparison to other two chemical agents.

Keywords: Histological Changes, Chemical Agent, 95% Ethanol, 4% Formaldehyde, 10% Silver Nitrate

INTRODUCTION

Vas deferens is the continuation of the epididymis ascending up on the medial side of it traverse the inguinal canal curving round inferior epigastitic reaching lateral wall of pelvis lying medial to obliterated umbilical artery, obturator, vesical nerves and vessels. It then crosses ureter reaching to its medial side running between bladder and seminal vesicle. Lastly passes down to and join with seminal vesicle to form ejaculatory duct which open in prostatic urethra. Before joining with seminal vesicle, vas dilates to form an ampulla of deferent duct on each side.

Histological changes: Ductus deferens has narrow irregular lumen and is made up of three coats-

1. External areolar coat.
2. Thick muscular coat which in greater part of the tube consists of two layers of unstriped muscles- an outer longitudinal and inner circular. At the commencement of duct, there is a third layer of longitudinal fibres placed between the circular stratum and muscle coat.
3. An internal or mucous coat which is arranged in longitudinal folds lined with columnar epithelium which is non-ciliated throughout the greater part of the tube. Many of the epithelial cells are secretary in nature.

Many scientists have been time to time trying to make it simpler for which various studies have been done. Kar and Kamboj[1] used silk thread as intra vas device for contraception. Schmidts et al[2] used electro-fulguration of surgically exposed vas and later he used electro-fulguration of cut end of vas in vasectomy and achieved better results. The incidence of antibody formation against sperms was also less in this procedure. Buersheke et al[3] kept working on bringing out a reversible mechanical technique as valvular prosthetic device fitted in vas which controls the flow of sperm and could be opened and closed as needed. Gupta AS et al[4] used tantalum clips and found that chances of haematoma and inflammation were less as compared to surgical vasectomy since procedure was less traumatising to vas. Albert et al.[5] found that the pre-operative irrigation of distal end of vas with Nitrofurazone 1 ml per mm kills sperms and thus sterility may be achieved at early hours in post vasectomy period.

Freeman C et al[6] was the first to introduce non-surgical technique of vasectomy by injection of sclerosing agent into vas deferens. He used 95% Ethanol, 10% Silver Nitrate, 4% Formaldehyde etc. and found encouraging results. Verma K et al[7] and Mishro et al [8] explained a technique by using a polymer. Polymer, co-polymer of styrene and maleic anhydride, was dissolved in dimethylsulphoxide and injected into vas deferens of rat. For the period of 180 days, it was an effective contraceptive in rats. Derrick et al[9] observed that in the first 2 to 3 weeks after vasectomy, spermatogenesis remains relatively accumulate in the tubules. Between the third and sixth weeks, we uniformly observed progressive spermatogenic arrest, with few spermatozoa and decreased spermatids.

Therefore aim of present study was to find out the histological changes in vas deferens after injection of various chemical agents as sclerosing agents as a method of male sterilization.

MATERIAL AND METHODS[10]

Present study was conducted in L.L.R Hospital, Kanpur in

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How to cite this article: Singh J, Prasad J, Khan S, Khan S. Histological Changes in Vas Deferens after Chemical Vasectomy. International Journal of Contemporary Medical Research 2020;7(11):K1-K5.

DOI: http://dx.doi.org/10.21276/ijcmr.2020.7.11.25

45 cases of Prostatectomy done for benign hyperplasia of prostate during the period of August 1982 to June 1983. Cases with history of urinary tract infection (UTI) or with genitor-urinary pathologies were excluded from the study. Mean age of cases ranged between 50-75 years. Cases were divided into three groups on the basis of different chemical agents:

1. Group A- 15 cases, 95% Ethanol.
2. Group B- 15 cases, 4% formaldehyde solution in water.
3. Group C- 15 cases, 10% Silver nitrate solution in water.

These chemical agents were injected percutaneously in the vas deferens, with a hope of obliteration resulting from inflammation and fibrosis of vas structures. Biopsy was taken 2-6 weeks after injection.

Part of the volunteer was prepared by shaving inguino-serotal region with upper part of thigh. Then patient was taken to operation theatre to maintain asepsis and avoid any complications. In every case, part was painted with savlon and diluted polyvinyl iodide (Betadine) and draped. Although this procedure does not require any anaesthesia but some patients may be apprehensive and their pain threshold may be 10%, they may require little xylocaine-1% as local anaesthetic-i.e. 1-2% in spermatic cord,0.25-0.5 cc at the point of injection in the vas. While carrying out the above study, majority did not require any anaesthesia and remained without any pain in post-injection period.

Fixation of VAS

Human vas runs from testes in the scrotum, in the cord to inguinal region. During this course since the scrotal skin is very thin, same can be palpated between thumb and index finger and can easily be brought very close to scrotal skin and thus can be made prominent. Since vas is situated on the posterior-lateral aspect of spermatic cord, approach from the posterior side of scrotum for fixing the vas would be convenient.

If by the above procedure fixation of vas has been difficult then we might fix the same by passing a 20 bore needle under the vas piercing through the scrotal skin. This would prevent the slipping of vas bad into the scrotum. But here we have got to use little local anaesthesia in order to avoid pain induced by needle prick. In yet other cases where above procedures have failed, fixation of vas may be done by Allis's/ Babeod's forceps.

Technique of injection of chemical into VAS

After the fixation of vas has been done, different chemicals of 95% alcohol, 4% formaldehyde, 10% silver nitrate were injected in different group of volunteers, separately. A 25 gauze needle is inserted in vas through the serotal skin, in retrograde fashion about 1" and away from testicular end and attempt was made to reach in the tissue of vas. For injection in vas tuberculin/insulin syringe was used and the total quantity of the chemical injected was roughly 0.1 to 0.5 ml in different trial groups as group A,B & C of 15 patient each. Each group is divided in 5 sub-groups of three each, which were given varying quantities of chemical i.e.0.1 to 0.5 ml. Soon after the injection, palpatory thickness of the vas increases if the chemical has really gone in.

In the subjects who were volunteers, right vas was injected with chemical while the left vas was injected with 0.9% saline i.e. normal saline which worked as control. Not more than four minutes were required for all injections including the anaesthetic to become effective. No significant discomfort was experienced after injection and patients could resume their work or activity. Soon after the injection, patients were given scrotal support in order to reduce the pain. No antibiotics were needed in post-injection period since all the chemicals which were used had strong antiseptic action. Mild analgesics have been prescribed (Whenever and wherever needed).

Biopsy and histology of VAS

Two to six weeks following injection, volunteer subjects were again taken to operation theatre for the biopsy of vas. It is taken on both sides i.e. left side (chemical) and right side (control), in order to compare. After pre-requisites for biopsy as part preparation etc in theatre, subject is made to lie supine with thighs wide apart for proper exposure of the scrotum. Strict aseptic precautions were taken to avoid sepsis.

1-2 cc of 1% xylocaine was given in spermatic cord and locally where incision was planned. Vas was palpated and brought to skin of the scrotum then fixed by Allis's forceps at its proximal and distal ends. 1 cm incision was given just over the prominent vas which just cut the skin and dartos muscle. Careful dissection was done and biopsy was taken and submitted for macroscopic examination where the palpable thickened area of biopsied vas was measured and recorded. Then the biopsy was transferred to already labelled vial containing A.F.A.(Acetic acid, formalin and alcohol) solution as preservative and fixative. In the same way, biopsy was taken from opposite side i.e. left side (control) and preserved. Biopsy put in AFA solution is allowed to remain there for 48-72 hrs for fixation and preservation after which it is subjected to embedding and section cutting. Sections of whole length are examined for changes. Thickness of the section varied from 4-7 microns and these sections were stained with (A) Haematoxylin and Eosine and (B) Tetrachrome and thus they were ready for histological study of vas.

RESULTS

In present study total no. of 45 cases was included (Biopsy of vas was done in all cases) and they were divided into three groups on the basis of various chemical agents as:-

1. Group A- 15 cases, 95% Ethanol.
2. Group B- 15 cases, 4% formaldehyde solution in water.
3. Group C- 15 cases, 10% Silver nitrate solution in water.

Table 1 show that optimum amount of sclerosing chemical agent to bring histological obliteration is 0.2-0.3 ml. Quantity more than this can cause spillage and peri vas leak.

Observations of table 2 reveal that the results achieved after chemical agents in vas for sclerosing has given quite satisfactory response. On histological examination the effect of 3 different chemicals appeared as-

Best results were achieved with 95% ethanol since out of 15

cases, 13 cases showed occlusion of vas. There were only two cases where the lumen was patent but definitely reduced in size. With formaldehyde and silver nitrate, results were good but not as good as 95% ethanol. Although, in all cases of each group, 89% cases showed blockade of lumen and 11% patent but reduced lumen. Maximum disruption of vas tissue was done by 95% ethanol and the density of chronic inflammatory cells was also much more than with other chemicals. However, quite satisfactory results were achieved with other two chemicals also. Cases showing patent lumen belong to the group, where less than 0.2 ml of chemical was injected which was not able to bring about the histological obliteration.

Observation of table 3 shows the effect of chemicals on

Agent	No. of Cases	Quantity (ml)	Length of vas obliterated	Control
95% Ethanol	3	0.1	Less than 1cm	Some inflammatory cells seen
	6	0.2-0.3	1.0-2.0 cm	
	6	0.4-0.5	2.0-3.0 cm	
4% Formaldehyde	3	0.1	Less than 1cm	Some inflammatory cells seen
	6	0.2-0.3	1.0-1.5 cm	
	6	0.4-0.5	1.5-2.5 cm	
10% Silver nitrate	3	0.1	Less than 1cm	Some inflammatory cells seen
	6	0.2-0.3	1.0-1.5 cm	
	6	0.4-0.5	2.0-3.0 cm	

Table-1: Histological obliteration of vas after injection of sclerosing chemical agent

Chemicals	Total no. of cases	No. of cases with total disruption of vas tissue and no lumen seen	No. of cases with lumen seen but completely occluded	No. of cases with lumen seen and partially occluded & shrunken	No. of cases with partially patent lumen
95% Ethanol	15	7	5	1	2
4% Formaldehyde	15	5	6	3	1
10% Silver Nitrate	15	4	7	2	2

Table-2: Showing chemical agents in relation to histological obliteration of vas deferens

Chemicals	Cases	Mucosa			Lamina Propria	Muscle layers
		Lumen		Epithelial cells		
95% Ethanol	15	R-3	N-0	Flattened, Distorted, replaced with scar tissue and inflammatory cells	Loaded with fibroblast and inflammatory cells	Full of fibroblast and chronic infla. cells
		B-5	F- All			
		A-7	D-12			
4% Formaldehyde	15	R-1	N-0	Flattened, Distorted, replaced with scar tissue and inflammatory cells	-do-	
		B-9	F-All			
		A-5	D-14			
10% Silver Nitrate	15	P-2	N-0	Flattened, Distorted, replaced with scar tissue and inflammatory cells	-do-	
		B-9	F-All			
		A-4	D-13			

R-Reduced, B-Blocked, A-Absent, N-Normal, F-Flattened, D-Disrupted

Table-3: Histological changes in human Vas after two to six weeks after injection of chemical sclerosing agents

Time Interval	95% Ethanol		4% Formaldehyde		10% Silver Nitrate	
	No. of Cases	Changes in Vas	No. of Cases	Changes in Vas	No. of Cases	Changes in Vas
2 Wks	9	Vas tissue replaced by scar tissue and chronic inflammatory cells	8	Vas tissue replaced by fibrous cells and chronic inflammatory cells	10	Vas tissue replaced by scar tissue and chronic inflammatory cells
4 Wks	4	Increased fibroblast and fibrous tissue seen	5	Increased fibroblastic activity seen	2	Increased fibroblastic activity seen
6 Wks	2	Changes seen as at 4 wks	2	Changes seen as at 4 wks	3	Changes seen as at 4 wks

Table-4: Showing time interval between injection of chemical agents and biopsy of Vas in relation to the histological changes

histology of vas deferens. Results reveal that 95% ethanol brings most satisfactory changes in vas, desired for obliteration. Seven shows complete destruction of vas tissue and replacement with chronic inflammatory and fibrous tissue. Five with chronic inflammatory changes and blocked shrunken lumen and three cases revealed narrowed but patent lumen. In 12 out of 15 cases with ethanol the epithelium was totally destroyed and disrupted and in all the cases the rest of the vas tissue was full of fibroblasts, fibrous tissue and chronic inflammatory cells.

With 4% formaldehyde and 10 % silver nitrate solution (on separate study on 15 patients each) results were almost same but slightly inferior to that of vas was found in 5 and 4 cases respectively. Changes in muscle part of vas were alike in all groups and cases.

Study of observations from table 4, reveals that the histological changes observed in biopsies of same group at 2 weeks were definitely sufficient for required obliteration but the amount of fibrous tissue and fibroblasts increased in cases where biopsy was taken at 4 or 6 wks intervals. Hence the density of the scarring increases to its maximum of 4 weeks then becomes stationary. Thus one concludes that the maximum histological changes occur latest by 4th week and then become stationary, since in biopsy at 6 weeks interval, in the same group, histological changes in vas were almost same as at 4 weeks.

DISCUSSION

Results of present study showed that cases in which less than 0.1 ml chemical was injected, did not show histological changes in vas required for obliteration. Optimum quantity of chemical for obliteration of vas is 0.2 ml-0.3 ml with more length of vas was destroyed with 10% silver nitrate solution. Results achieved after the histopathological examination of the macroscopically thickened vas resulting from different chemical injections were carefully observed for obliteration. In all 89% cases revealed blockage and 11% just reduction in human size (i.e. with patient human). Previous studies do not indicate towards such a high rate of failure. Failure in our study may be because of two reasons: (1) the cases that were injected with sub-optimal dose of 0.1 ml, were included in study technique requires skill with expertise and experience hand comes through practice. To eliminate the later error starting cases that were just injected for the purpose of practice were not included in the study.

However, our results were quite satisfactory. Best results were achieved with 95% Ethanol injection. Out of the 15 patients of group- A, 7 revealed complete disruption of the vas –deferens tissue which was totally replaced by the fibroblasts, fibrous tissue, chronic inflammatory cells and the lumen in the section was totally invisible in next five cases of same group. Lumen was visible but greatly shrunken so much so that the flattened folds and epithelium was closely approximating from all sides occluding lumen. A single case had partially occluded lumen and the other two cases had patent lumen from sub-group of 0.1 ml injection.

4% formaldehyde also gave good results since total disruption was seen in 5 cases while other 5 revealed blocked lumen. There was partial occlusion of lumen with considerable reduction in size in three cases and a single case showed patency. The only adverse effect of formaldehyde was that it was associated with complications like epididymo-orchitis, fever etc sometimes.

10% silver nitrate solution in distilled water also gave encouraging results. Four out of 15 cases showed complete obliteration with no lumen, 7 had visible obliterated lumen and 2 were partially blocked. Rest 2 cases showed patency of lumen.

As regards the permanency of changes, histological changes were sufficient to bring about the obstruction of the lumen of vas but the results at the 4 weeks interval showed such fibrous tissue with scarring and then became stationary. Histological findings of 6 weeks interval biopsy were almost similar to that of 4 weeks reports. This concludes that the expected results may be achieved at 2 weeks only but sure shot obliteration would occur by end of 4 weeks and thereafter remains as such. However, some cases must be subjected to biopsy after few months interval after injection, so as to get the scientific evidence of persistent obliteration. However, in our study, no biopsy was done after 6 weeks and a question of spontaneous recanalisation after sometime may arise but the histological changes occurring are biologically irreversible, considering the nature of replacing tissue. Still such cases should be studied in follow up in future trials which may give confirmed idea about spontaneous recanalisation, at varying intervals.

CONCLUSION

From the results of present study, it can be concluded that optimum amount of sclerosing chemical agent to bring histological obliteration is 0.2-0.3 ml. Chemical agents in vas for sclerosing has given quite satisfactory response with 95% ethanol showing most satisfactory changes in vas, desired for obliteration. Maximum histological changes occur latest by 4th week and then become stationary, since in biopsy at 6 weeks interval, in the same group, histological changes in vas were almost same as at 4 weeks.

Therefore it can be concluded that best results are achieved with 95% ethanol in comparison to other two chemical agents. In present study there is evaluation of sclerosing effect of various chemicals on basis of histological examination but to establish real infertility, semen examination for sperm count should also be done simultaneously. Present study has conclusively demonstrated the potential advantages of vasoinjection opposed to vasectomy. Limitation of present study is small sample size therefore there is need for further studies with large sample size for l for prediction of success or complication rates.

REFERENCES

1. Kar and Kamboj. Spermatozoa disintegration in rats with an intra vas deferens device. Ind. J. Exper. No. 1961; 2:240.
2. Schmidts SS. Technic and complications of electrical vasectomy. Am. J. Fert. & Ster.1967;17:167.

3. Buersheke, Burns M,Hofer M et al. Development of reversible vas occlusive device Am. J. Fert. & Ster.1975;26:40.
4. Gupta, AS, Kothari LK. Vas occlusion by Tantacum Clips, its comparison with conventional vasectomy in man, reliability, reversibility and complications. Am. J. Fert & Ster.1977; 28:186.
5. Albert, Peter S, Seebode J, Nitrofurasone, Vas irrigation as an adjunct in vasectomy: Am. J. Fert & Ster.1978; 29:442.
6. Freeman C, Coffey DB. Sterility induced by ethanol injection into the vas deferens. International J.Fert & Ster.1973; 18:129.
7. Verma K, Misro MM, Singh H et al. Histology of the rat vas deferens after injection of a non-occlusive chemical contraceptive. J. Reprod. Fert. 1981;63:539-42.
8. Mishro MM, Guha SK, Singh H, Mahajan S et al. Injectable non-occlusive chemical contraception in the male-I. Contraception. 1979;20:467-73.
9. Derrick FC, Glover WL, Kanjuparamban Z et al. Histologic Changes in the Seminiferous Tubules after Vasectomy. Fertility and Sterility.1974;25: 649-58.
10. Singh J, Prasad J, Khan S et al. Role of Chemical Vasectomy by using Various Chemical Agents as a Method of Male Sterilization. IJCMR.2020;7: G1-8.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 19-10-2020; **Accepted:** 08-11-2020; **Published:** 30-11-2020