

Comparison of the depth of tissue necrosis between double-freeze and single-freeze nitrous oxide-based cryotherapy

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ABSTRACT

Background: Cryotherapy is one the methods of treating cervical premalignant lesions. It is particularly suitable for low-resource countries because of it is relative cheaper, has low cost of maintenance, ease of use and that does not require electricity which is in short supply in many rural areas of developing countries where the incidence and mortality from cervical cancer is very high. In this study we compared single and double freezing on the cervixes of women admitted for hysterectomy for benign conditions using Nitrous-based cryotherapy. **Materials and Methods:** Patients admitted for elective hysterectomy for benign gynaecological conditions were randomized into two arms. The first group had single freeze cryotherapy while the second arm received double freeze cryotherapy. The cervixes were examined 24 hours later to determine the depth of tissue necrosis. **Results:** In this comparative study, the depth of tissue necrosis was deeper with double freeze compared with single freeze. Also in both arms, the depth of necrosis was deeper on anterior lips than on posterior lips of the cervix. **Conclusion:** Double freeze technique achieve more depth of tissue necrosis than single-freeze on both anterior and posterior lips of the cervix.

Key words: Cervical premalignant lesions, cryotherapy, nitrous oxide

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INTRODUCTION

Cervical cancer is the second leading cause of cancer deaths among women, and the most common gynecological malignancy in developing countries.¹ Worldwide, about 500,000 new cases are diagnosed every year with approximately 274,000 deaths.¹ Over 80% of new cases and approximately 85% of deaths occur in developing countries of the world.^{2,3} In the developed countries, there has been a progressive decline in incidence and mortality from cervical cancer.⁴ This decline is attributed to organized screening program for the detection of premalignant lesions of the cervix, treatment, and adequate follow-up of the detected cases.⁵

The method of cervical cancer screening introduced by Papanicolaou several years ago and currently called conventional cytology is still the mainstay of cervical cancer screening program in most countries of the world.^{4,5} Other methods of screening include the visual inspection with acetic acid and the oncogenic HPV DNA testing.^{6,7}

It should, however, be noted that screening alone does not lead to reduction in the mortality or incidence of cervical cancer. To achieve the desired results, screening must be followed by adequate and effective treatment of the detected cases. Treatment of cervical premalignant lesions

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could either be performed by ablation or excision. Ablation involves destruction *in situ* of entire transformation zone and thus the abnormal areas, whereas excisional methods involve the removal of the abnormal areas.^{8,9} The main difference between the two methods of treatment is that tissues are available for histological examination in excisional methods. For carefully selected patients, the outcomes of treatment are comparable between the two treatment mortalities; however, cryotherapy is associated with lower cost.¹⁰

One of the ablative methods is cryotherapy which was introduced to reduce the morbidities associated with cone biopsies and hysterectomy used to treat such lesions.^{11,12}

Cryotherapy is preferred in many developing countries because of it is cheap compared with other treatment modalities for the treatment of cervical premalignant lesions. It is easy to use, easily learnt, and does not depend on electricity which is in short supply in many developing countries.

In cryotherapy, compressed air is used to destroy abnormal tissues by lowering the temperature of the affected tissues. Most cryosurgical instruments use either nitrous oxide (freezing point of -89°C) or carbon dioxide (freezing point of -65°C). Cellular death occurs at a temperature of approximately -20°C .¹³ Cryosurgery produces severe biochemical and biophysical changes resulting in coagulation of the affected tissues. Rupture of the cell wall occurs with the formation of intracellular and extracellular ice crystals. Avascular necrosis is produced by circulatory compromise due to capillary obstruction and stasis.¹⁴

The critical aspect of cryotherapy is that the extent of spread of the ice ball formed at the margins of the cryotips is more important than the duration of freezing.

The formation of the ice ball usually occurs within 2 min of starting the cryotherapy if there is an adequate supply of refrigerant gas. To be effective in destroying premalignant lesions on the cervix, the depth of cryonecrosis should extend approximately to 4–5 mm and theoretically should destroy any intraepithelial neoplastic process and also for diseases extending into the endocervical glands on the portio.¹⁵ One of the controversies among clinicians is the method of cryotherapy needed to achieve cryonecrosis sufficiently to treat cervical premalignant lesions.

Cryotherapy can be used in two modalities; single-freeze and double-freeze. The double-freeze involves freezing of the transformation zone for 3 min, thawing for 5 min, and refreezing again for another 3 min to complete the double-freeze cycle. The other technique is the single-freeze method which involves freezing for only 3 min. Though double-freeze is preferred by many clinicians, this is backed by few scientific facts.

There is also paucity on information comparing the depth of freezing between the anterior and posterior lips of the cervix. The cervix projecting into the vagina is slightly angulated downward and backward. Thus, the cryoprobe may not be uniformly in contact with the ectocervix. This may affect the degree of freezing between the anterior and posterior lips of the cervix.

This study aims to compare the depth of freezing between single-freeze and double-freeze techniques using nitrous oxide-based cryotherapy.

This study also aims to compare the depth of freezing between anterior and posterior lips of the cervix following cryotherapy.

MATERIALS AND METHODS

This is a single-blind prospective study conducted on patients admitted into the gynecological ward for elective hysterectomy for benign gynecological conditions. Approval for the study was obtained from the Institution's Ethical Committee.

Informed consent was obtained from eligible patients before being randomized into two arms; double-freeze and single-freeze arms of the study. Randomization was carried out on sixty women using computer-generated table of random numbers.

Twenty-four hours before hysterectomy, the cryoablation of the cervix was carried out using an appropriate cryoprobe. In the double-freeze arm, cryotherapy was applied for 3 min, followed by 5 min rest, and a reapplication for another 3 min. Those, in the single-freeze arm, had only 3 min of cryotherapy.

Following hysterectomy, the anterior lip of the cervix was tagged with silk suture for proper orientation and sent to the pathologist who was blinded to which arm the specimens belonged; the pathologist then excised the cervix from the uterus and bisected at the 3 and 9 o'clock positions to separate the anterior and the posterior lips. Both pieces were fixed in formalin for 24 h; multiple sections were obtained from each one of the lips. The microscopic evaluation was focused on the measurement of the depth of necrosis by superimposing a micrometer in the $\times 10$ eyepiece. Multiple measurements were taken and only the deepest area of necrosis was recorded. The criterion to determine the deepest level of necrosis was based on the destruction of the glandular epithelium in the gland crypt in the stroma or destruction of the endothelium of the stromal blood vessels.

RESULTS

The women in both arms of the study are similar with respect to age and parity with the mean age being 46 and 45 years.

The mean depth of necrosis on the anterior lips for the double-freeze technique was 5.2 mm and 4.2 mm for the single-freeze method [Table 1]. On the posterior lip of the cervix, the mean depth of necrosis was 4.9 mm and 3.8 mm for the double- and single-freeze technique, respectively ($P = 0.000$) [Table 2]. The difference between the mean depths of tissue necrosis between the anterior and posterior lips in the double-freeze arm was 0.3 mm ($P = 0.003$) and in the single-freeze arm was 0.4 mm ($P = 0.029$). The double-freeze method achieved the critical depth of tissue necrosis of ≥ 4.8 mm on the anterior lip in 87% of cases and only 73% cases on the posterior lip. In the single-freeze arm, this critical depth was achieved in 37% of cases on the anterior lip and 20% of cases on the posterior lip.

DISCUSSION

Cryotherapy is one of the most cost-effective means of treating cervical precancerous lesions in the developing countries because it can be carried out as an office procedure, requiring neither anesthesia nor electricity and added to this is the fact that only simple and relatively cheap equipment is required. This is particularly important in low-resource settings where power supply is a big challenge. It also has the advantage of been useful in the “see-and-treat” protocol particularly when visual inspection with acetic acid is used for mass

screening program in low-resource settings.^{16,17} This study showed that the mean depth of tissue necrosis using the double-freeze method was better on both lips of the cervix compared to the single-freeze method. On the anterior lip, it was 5.2 mm in the double-freeze arm compared to 4.2 mm in the single-freeze arm ($P = 0.000$). Similarly, on the posterior lip, the depth of tissue necrosis achieved by the double-freeze and single-freeze was 4.9 mm and 3.8 mm, respectively ($P = 0.000$). A freezing depth of 4.9 mm is considered adequate to destroy premalignant lesions extending even up to the endocervical glands on the portion.

In a prospective study involving 142 women, Schantz and Thormann showed that the double-freeze technique was significantly more effective than the single-freeze technique. In that study, after a follow-up period of more than 2 years, one of the factors associated with recurrence was single-freeze method.¹⁸

The most severe form of cervical premalignant lesions; CIN 3 can extend into the glandular crypt of the epithelium; a depth of necrosis of at least 3.5 mm is, therefore, needed to eradicate 95% of cases and 4.8 mm to eradicate more than 99% of cases.¹⁹ The mean depth of tissue necrosis on the anterior lip of the cervix was similar to the findings of Mariategui *et al.* who in a comparative study between nitrous oxide and carbon dioxide showed that the mean depth of necrosis by nitrous oxide was 5.3 mm, compared with 5.0 mm on the posterior lip.¹⁷

To adequately treat cervical premalignant lesions, it is important that the depth of tissue necrosis achieved by cryotherapy should be at least 4.8 mm.¹⁹ This was achieved in 87% and 73% cases of anterior and posterior lips, respectively, using the double-freeze method. On the other hand, using the single-freeze approach, adequate treatment was achieved in only 37% and 20% of cases in anterior and posterior lips, respectively. This may partially explain the reasons apart the size of the lesions why cryotherapy may not be as effective as loop electrosurgical excision procedure (LEEP).

Reasons for the difference in freezing between the anterior and posterior lips may be due to the fact that the parts that are in closer contact with the cryoprobe receives the highest freezing, causing deeper necrosis, and this was the anterior lip in most cases. The implication of this finding is that most lesions on the posterior lip would be incompletely treated using cryotherapy either double or single-freeze. It might, therefore, be more appropriate to use other modalities of treatment such as LEEP for lesions on the posterior lip of the cervix.

Even though this study used women with normal epithelium, we do not expect any significant difference in the presence of dysplasia.¹³

Table 1: Depth of tissue necrosis anterior lips of the cervix

	Double-freeze	Single-freeze
n	30	30
Mean (mm)	5.2	4.2
Median (mm)	5.2	4.2
SD (mm)	0.83	0.64
SE (mm)	0.15	0.12
Minimum (mm)	3.5	3.0
Maximum (mm)	7.0	5.0
n ≥ 4.8 mm	26	11
Percentage ≥ 4.8 mm	87	37

SD – Standard deviation; SE – Standard error

Table 2: Depth of tissue necrosis posterior lips of the cervix

	Double-freeze	Single-freeze
n	30	30
Mean (mm)	4.9	3.8
Median (mm)	5.0	3.8
SD (mm)	0.85	0.73
SE (mm)	0.15	0.13
Minimum (mm)	3.2	2.6
Maximum (mm)	6.9	4.9
n ≥ 4.8 mm	22	6
Percentage ≥ 4.8 mm	73	20

SD – Standard deviation; SE – Standard error

CONCLUSION

Double-freeze nitrous oxide-based cryotherapy achieved more depth of tissue necrosis on both lips of the uterine cervix than the single-freeze technique. The double-freeze method should, therefore, be the preferred modality when cryotherapy is indicated for the treatment of cervical precancerous lesions.

Better depths of tissue necrosis were also achieved on the anterior lip than on the posterior lip in both arms of the study. Double-freeze cryotherapy may, therefore, be more appropriate in treating cervical precancerous lesions on the anterior lip than those on the posterior lip of the cervix. Wide lesions on the posterior lip might need other modalities of treatment to achieve a total cure. The need for follow-up should also be emphasized to women who had cryotherapy, as total cure may not always be achieved.

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Conflicts of interest

There are no conflicts of interest.

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