WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 1718-1729. Research Article ISSN 2277– 7105

COMPARATIVE STUDY OF VARIOUS SOLUTIONS FOR ENDOSCOPIC SUBMUCOSAL INJECTION: A PORCINE STOMACH MODEL

Kitti Chuenyong*[1] and Kanokpoj Chanpiwat[1]

[1]Gastroenterology Division, Internal Medicine Department. Rajavithi Hospital, College of Medicine, Rangsit University, Bangkok, Thailand.

Article Received on
10 March 2020,
Revised on 31 March 2020,
Accepted on 21 April 2020
DOI: 10.20959/wjpr20205-17435

***Corresponding Author**
Dr. Kitti Chuenyong
Gastroenterology Division, Internal Medicine Department. Rajavithi Hospital, College of Medicine, Rangsit University, Bangkok, Thailand.

ABSTRACT

Purpose: To study the mean duration of mucosal elevation (DME) of four different solutions in an ex-vivo porcine stomach model. Inter-investigator variations in DME values were also investigated. **Patients and Methods:** Four injectates, namely NSS, glyceol, 25% glucose and 50% glucose, were used. A total volume of 1 mL of each solution was injected into one 4x4 cm square gastric piece. The injections were administered by two investigators independently, and VDO recordings by digital camera were made at the time of each injection. Durations of mucosal elevation (DME) were recorded and analyzed. **Results:** A total of 10 specimens per solution per investigator were utilised, so that 80 pieces of porcine gastric pieces were used in all. The DME of NSS, glyceol, 25% glucose and 50% glucose were 103.26, 76.87, 66.78 and 48.49 min respectively. Only NSS and 50% glucose were significantly different. No difference was detected in DME values of the two investigators. **Conclusion:** NSS had the longest DME in our ex-vivo porcine stomach model, and this was contrary to the findings of some in-vivo studies. We found that the ex-vivo porcine stomach models were not appropriate for comparing the duration of the submucosal cushion from various injectates; an in-vivo live animal model would be better.

KEYWORDS: Submucosal injection, fluid cushion, endoscopic mucosal resection, polypectomy, porcine stomach, injectate.

INTRODUCTION

Polypectomy via endoscopy is a common gastro-intestinal procedure for removal of polyps. Most polypectomies are not difficult, so that gastroenterologists can perform them alone. In the case of colonic polyp, polypectomy has demonstrated benefits in terms of reduced mortality rates in colorectal adenocarcinoma.[1,2] Recently, new recommendations have been made for management of sessile polyps in the form of hot snare polypectomy (HSP) for polyps between 10 to 20 mm. in diameter and endoscopic mucosal resection (EMR) for polyps 20 mm. or more in diameter.[3] Submucosal injection before HSP or EMR is essential to reduce complications from thermal injury to the muscular layer and also to facilitate complete resection of the polyp, known as en-bloc resection.[4] Many types of liquid substances are employed for submucosal injection. Normal saline (NSS) is the most commonly-injected solution; however, it is not safe in cases of HSP because it has a very short duration of submucosal cushion after the injection, and other agents which can make mucosal lift persist for longer periods are more appropriate for this procedure.[5] Sodium hyaluronate (SH) is the best and most appropriate solution for mucosal lift before performing endoscopic submucosal dissection (ESD)[6,7]; however, it is very expensive and therefore not practical for routine use in Thailand. Other solutions which have been demonstrated in research to be better than NSS include dextrose or glucose solution and glyceol.[4,8,9]

Although there have been meta-analyses and systematic reviews comparing many kinds of injectate, the results are still controversial[5,10]; furthermore, most data used in these studies were very different and heterogeneous, leading to poor reliability of results.

One study performed in 2002 in living pigs showed that NSS had disappearance times shorter than those of 50% dextrose, 10% glycerine and hyaluronic acid.[11] The precise meaning of disappearance time was uncertain because it was determined during endoscopy, thereby resulting in varying angulation between the scope and submucosal cushions.

Two other studies used fresh porcine stomach pieces to compare various solution types. The first one in 2010[12] showed that SH was the best solution for maintaining mucosal elevation, and that other agents were also better than normal saline. The other such study, published in 2012[13], compared mucosal-elevating times of NSS, glyceol, SH, hydroxyethyl starch, serum, plasma and whole blood, and whole blood showed the longest-lasting mucosal elevation. Although blood component is cheap, using it for submucosal injection before doing EMR or polypectomy is questionable in terms of its cost-worthiness in real practice.

Hypertonic glucose solution is cheap and readily available and has been found to be effective in submucosal injection, but it has never been compared head to head with NSS. Our study was conducted to compare the mucosal elevation times of hypertonic glucose solution with those of glyceol and NSS.

MATERIAL AND METHODS

This was an experimental study, conducted at the Gastrointestinal Unit, Rajavithi Hospital, from April 2018 to April 2019. A total 80 pieces of fresh porcine stomach were used. The solutions used in this study were NSS, glyceol, 25% glucose and 50% glucose; we used two different concentrations of glucose solution in order to compare the differences in their effect on mucosal elevation time.

The primary objective of our study was to compare the mean duration of mucosal elevation (DME) of four different solutions: NSS, glyceol, 25% glucose and 50% glucose using an ex-vivo porcine stomach model. The secondary objective was to explore the effect of injectors on mean DME. Two investigators administered the injections in parallel.

Materials

- Porcine stomach 4x4 cm^2
- Foam sheets
- Solutions: normal saline, glyceol, 25% glucose and 50% glucose
- 3 mL disposable syringes
- 24 gauge needles
- Push pins
- Scaling background sheets
- Digital camera

Porcine stomach preparation

All porcine gastric pieces were collected from the same place and were collected fresh (less than 4 hours) without freezing in order to keep tissue quality as close as possible to that of an in-vivo model. After cleansing, the porcine stomachs were cut into 4x4 cm^2 pieces. The porcine gastric parts were fixed to foam sheets by push pins before the experimental sets.

Injectate preparation

The injectates used in our study were NSS, glyceol, 25% glucose and 50% glucose. All were aspirated into 3 mL plastic syringes, and the injection sequences were randomly arranged by a nurse in our team. Ten syringes of each solution, in total 40 syringes, were labelled with codes. The two investigators were blinded until the final results had been completely interpreted.

Submucosal injection technique and experimental environment

All injections were made by two doctors on separate days; however, the environment of our experimental room was maintained as identical as possible on each day in terms of room temperature, experimental time and video recording system. All experiments were performed in the morning in order to have porcine specimens which were as fresh as possible. Each syringe was filled with one of the four injectates and then closed by 24 gauge injecting needles before being delivered to the doctors. The needle and syringe were aligned in oblique direction to the surface of porcine gastric specimens with angulation of around 30 degrees to the horizontal line. The needle tips were placed down to the submucosal layer. One mL solutions were injected in all specimens, after which submucosal cushions were formed in a dome-shaped mass.

Video recording and experimental system design

The experimental system, including porcine stomach piece, was fixed on a foam sheet with a digital camera and background sheet. The background sheet showed scaling line and the zero line was adjusted to the same level as the specimen's surface. Video recordings were carried out by the same person, and all videos were recorded onto an external hard disc.

Data extraction and analysis

All 80 video files from the two investigators were reviewed and interpreted at the end of the study. The investigators were still blinded to the solution types at this stage to prevent bias in data extraction. The injectants were labelled as 1, 2, 3 and 4. All video files were extracted by the two doctors independently, and duration times of submucosal cushion maintenance were extracted from each specimen.

Definition

Duration of mucosal elevation (DME) in our study was defined as the period of time from the administration of injection to the point in time when mucosal elevation disappeared or remained in a steady state for a long period.

Statistical analysis

The seventeenth edition of the statistical package for the social sciences (SPSS) program was used for data collection and analysis in this study.

Descriptive statistics

Categorical data were reported as percent (%), while continuous data were presented as mean ± standard deviation (SD) if they were parametric data or median (25th–75th IQ) if they were nonparametric data.

Inferential statistics

In order to compare categorical data, Chi-square test (parametric) or Fishers' exact test (nonparametric) were used.

To compare continuous data which had no correlation, student T-test was used in the case of parametric data and Man-Whitney U-test was used for nonparametric data.

To interpret inter-observer agreement of duration of mucosal elevation between the two investigators, Pearson's correlation or Kappa statistics were used.

A p-value < 0.05 was deemed statistically significant.

Sample size calculation

For sample size calculation in this study, the comparison of two means of Bernard R 2000 was used.

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 x(\sigma_1^{\ 2} + \sigma_2^{\ 2})}{(\mu_1 - \mu_2)^2}$$

Where:

n = sample size

α = probability of type I error

= 0.05 (2-sided)

$Z_{\alpha/2} = Z_{0.025} = 1.96$

$1-\beta$ = power (set as 80%), Z_β or $Z_{0.2}$ = 0.842

σ = common standard variation of population substituted by standard deviation (SD)

μ_1, μ_2 = true (population) mean in group 1 and 2 respectively.

In this study we used the mean and standard variation from the study of Conio M which showed mean duration of mucosal elevation (DME) of normal saline of 2.6 (±0.6) min and DME of glyceol of 5.2 (±2.6) min respectively. With regard to sample size calculation, a total of 10 samples per solution type per investigator were necessary, so that a total of 80 pieces of porcine gastric specimens were required.

Descriptive results of continuous variables were expressed as mean ± SD and median (25^{th}–75^{th} IQ). Chi-square tests and One-Way Anova were used to compare categorical and continuous variables in each grading group, and a *p*-value of less than 0.05 was set for statistical significance.

RESULTS

In our study, 80 pieces of porcine gastric pieces were successfully collected in accordance with the sample size calculation mentioned above. The DME data demonstrated a parametric distribution. As mentioned above, all injectants were blinded and randomized by the nurse, and all solutions were labelled as 1 to 4 (Table 1). The seal was opened after data extraction was completed.

Regarding the primary objective, the DME of the 4 kinds of injectate were as follows: NSS had the longest mean DME of 103.26 (±12.95) min, glyceol of 76.87 (±11.26) min, 25% glucose of 66.78 (±12.63) min and 50% glucose of 48.49 (±9.18) min. The *p*-value of the differences between all pairs was 0.012 and indicated that at least one pair had a statistically significant difference (Table 1).

The differences, pair by pair, of all 4 injectants were analyzed and showed that NSS had mean DME significantly longer than 50% glucose (p = 0.015) while comparison of mean DME of all other pairs showed no statistical significance: NSS had longer DME than glyceol (p = 0.464), and 25% glucose (p = 0.185). Glyceol showed mean DME longer than 25% glucose (p = 0.944) and also longer than 50% glucose (p = 0.399). Finally, 25% glucose had a longer mean DME than 50% glucose (p = 0.743) (Table 1 and Figure 1).

With regard to the secondary outcome, the influence of injector on the mean DME, the DME of the two investigators were recorded separately and were then compared. We found that the mean DME from the two investigators correlated well. The mean DME of all solutions were not significantly different between the two investigators: 50% glucose had 43.8 vs. 53.2 min ($p = 0.62$); glyceol had 65.3 vs 88.5 min ($p = 0.316$); NSS displayed 93.3 vs. 113.2 min ($p = 0.456$); and 25% glucose showed 67.1 vs. 66.5 min ($p = 0.984$) from first and second injector respectively (Figure 2).

As mentioned above, to minimize bias of data extraction from the video file, the two investigators independently interpreted them. The DME time between the two investigators revealed some differences, and we therefore used the mean DME extracted by the two investigators for statistical analysis. Although the mean DME values extracted from the video recording varied a little, the differences were not significant (the mean DME interpreted by investigator A was 85.8 min while that of investigator B was 60.65 min). All DME times extracted by the two investigators were analyzed using Pearson Correlation which revealed that the DME interpreted by the two investigators correlated well ($r = 0.73$) (Figure 3). As a result, we concluded that the DME in our study was not influenced by the interpreters.

Table 1: Mean Duration of Mucosal Elevation among Four Injectates.

Type of injectates	N	Mean DME (minute)	SD	*p* value for all group difference
1. 50% Glucose	20	48.49	9.18	0.012
2. Glyceol	20	76.87	11.26	
3. NSS	20	103.26	12.95	
4. 25% Glucose	20	66.79	12.63	
Total	80	73.85	6.11	

Abbreviations: DME, duration of mucosal elevation; NSS, normal saline.

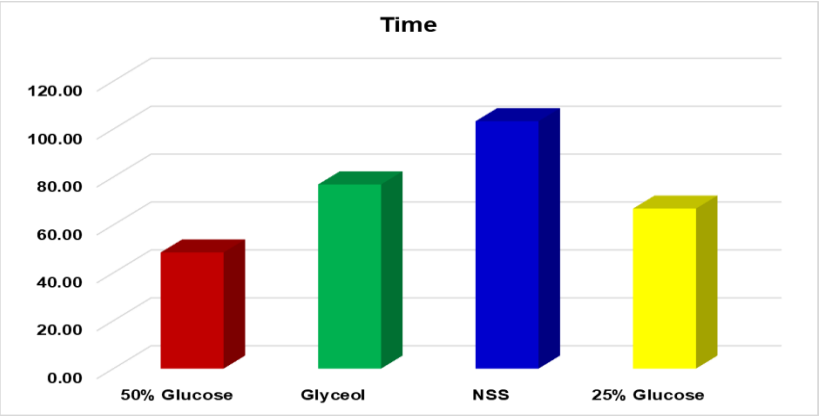

Figure 1: Pair by pair comparison of the mean DME of the 4 studying injectates.

Note: Only NSS vs 50% Glucose had statistical diffence

Abbreviations: DME, duration of mucosal elevation; NSS, normal saline.

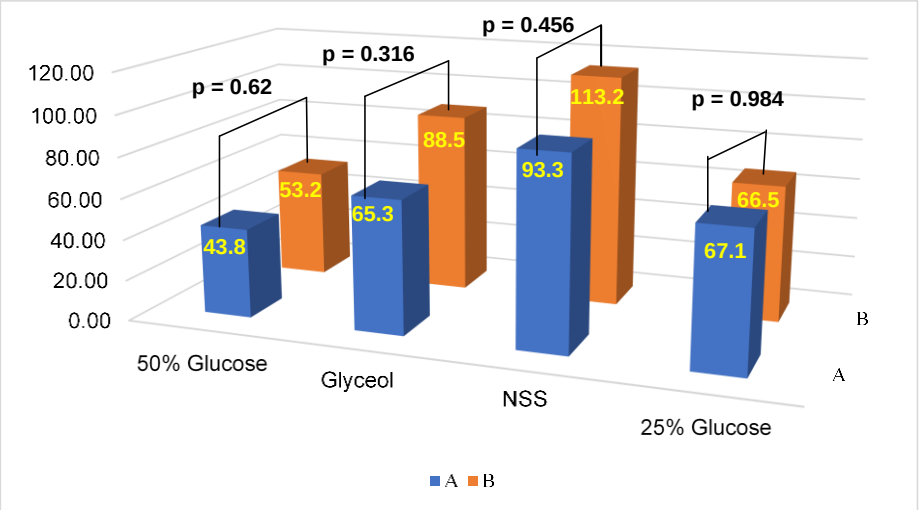

Figure 2: Comparison of the mean DME of the four injectates injected by both investigators.

Abbreviations: DME, duration of mucosal elevation; NSS, normal saline.

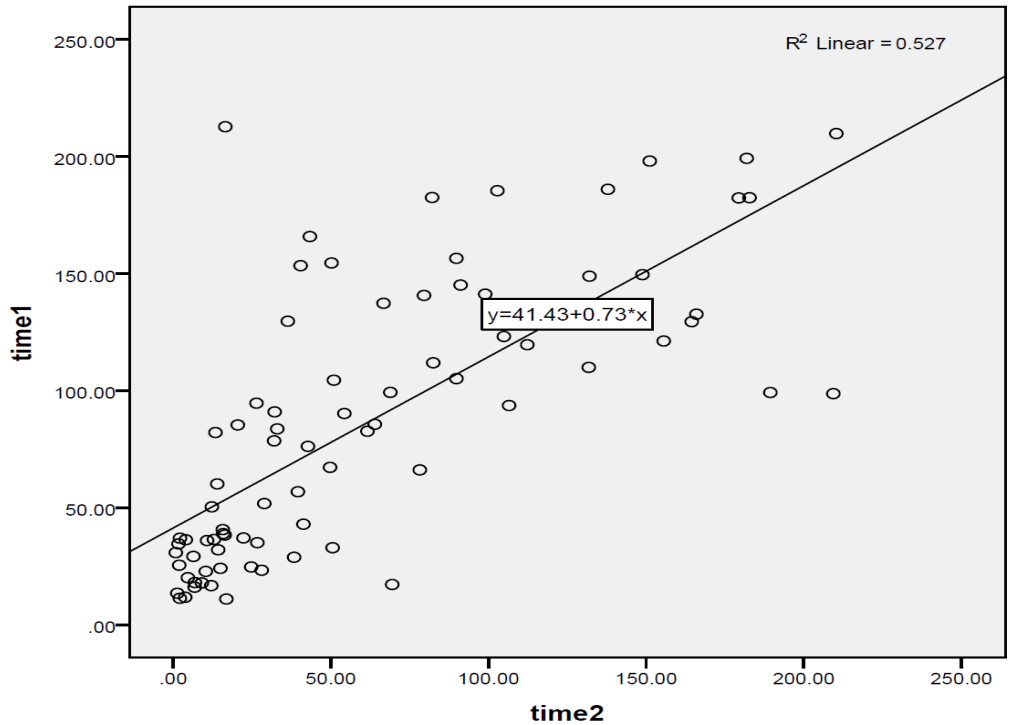

Figure 3: The Pearson Correlation plot to compare all mean DME interpreted from the video clips by both investigators.

Note: The r = 0.73 indicated that the mean DME extracted by both investigators were well correlated.

DISCUSSION

Our study was the second, after that of Polymeros in 2010[12], and the first in Asia, to investigate the use of different injectates in an ex-vivo porcine gastric model. There were many strong points in this study: first, all solutions in our study were cheap and very practical; second, the investigators were blinded to injectate types in order to minimize bias; third, the data extraction processes were independently performed by the two investigators in order to check the validity of the process; and lastly, we organized standard instrument settings to make sure that the visualizations were set at the same standard.

Solutions of 50% dextrose had previously been compared with other agents in only two in-vivo studies, and ours was the first one to compare it to NSS in ex-vivo porcine gastric pieces. Conio M found that 50% glucose was better than NSS in living pigs[11], while another two studies showed the advantages of 50% glucose over other injectates using submucosal cushion prior to endoscopic submucosal resection because it facilitated complete resection; however, the authors did not compare DME values.[8,9] Although two previous studies were performed in ex-vivo porcine stomach models[12,13], they did not use 50% glucose in their comparisons. After our experience of using 50% glucose solution for a submucosal cushion before performing EMR, we agreed with the findings of the study of Conio[11], which stated that it has much longer duration of mucosal elevation than NSS. This excellent outcome was also found in 25% glucose solution, and we therefore conducted the current study to compare both concentrations of hypertonic glucose with NSS in the ex-vivo model.

The main finding of our study (Table 1 and Figure 1) was that NSS showed the best DME; in particular, it had a statistically significantly longer DME than that of 50% glucose. This finding was different from those of many previous studies including one involving endoscopy in human EMR[8,9] and live porcine models[11], which demonstrated that 50% dextrose was superior to NSS in providing longer duration of submucosal cushion. We analyzed the reasons why NSS had longer mean DME than that of both Glyceol and hypertonic glucose solution, and we found that it was because of incorrect interpretation of the end of mucosal elevating time. We found that all of the hypertonic solutions were able to maintain elevation for many hours, but towards the end there was mild swelling above the zero line and it remained stable, so the authors interpreted this as the end point; on the other hand, NSS

showed declining elevation to the baseline at zero level, and therefore the DME should have been be much longer. Another reason was that although we selected only fresh ex-vivo gastric parts, they were totally different from living stomach tissue. We compared mean DME of NSS in our study which was about 103.26 min to that found in the study of Conio[11] in which it was only about 2-3 min. Clearly, this evidence supports our conclusion that ex-vivo gastric tissue is not a good model for studying the duration of submucosal cushion of injectates.

Our research was the first determine the influence of injectors on mean DME (Figure 2). We found that although the mean DME from the two investigators were different, the figures were not statistically significant. The variances in DME values may have been due to the depth of the injection and the technique used. The mean DME of each injectate from the two injectors were similar; for example, NSS had the longest DME while 50% glucose had the shortest DME. We concluded that there was no statistical significance in variations between the two investigators' measurements.

Another strong point of the current study is that it was the first one to conduct separate performance of data extraction from the video file by two investigators in order to study the variations in DME during data extraction. We found good correlation of DME values extracted by the two investigators (Figure 3); however, there were many points where data showed some differences because of the unclear end point as mentioned above.

Polymeros D et al[12] compared the duration of mucosal elevation among various injectates in a porcine gastric model. They reported that sodium hyaluronate had the longest elevated time of about 41 min while NSS had the shortest at around 12 min. Our study found much longer DME values, and this may have been due to differences in the definition of the end point. H Al-Tae O et al[13] also used an ex-vivo porcine gastric model to compare the rate of decline of the submucosal cushion of NSS and other agents including hyaluronate; however, their study did not focus on the duration of mucosal elevation. We did not use the rate of cushion absorption because we felt it was not easy to measure the height of the cushion during the video recording; furthermore, we found that the submucosal cushion height varied depending on the depth of needle puncture, and that if it was too deep, there would be lower cushion elevation.

The last strength of our study was that the investigators were blinded to the type of injectates used, both during injection administration and data extraction, thereby minimizing bias, and the results of our study were reliable. Although the results of this research cannot be applied to assess the quality of each substance in submucosal injection in in-vivo studies, it established that an ex-vivo porcine gastric model is not appropriate for use in investigations into the mucosal elevation qualities of injectates. While the study of Conio M[11], which used live animals, was appropriate for this type of research, more standard instrumentation systems are required to measure DME.

CONCLUSION

In the current study, NSS had the longest duration of mucosal elevation, while 50% glucose solution had the shortest. The results were contrary to the findings of research which used live animals, and also to daily practice in endoscopic procedure, because the ex-vivo porcine tissue quality led to delayed absorption of the submucosal cushion and an unclear time of return to the baseline. In the future, we suggest that any study aimed at comparing injectates for submucosal cushion creation should use live animal models.

ACKNOWLEDGMENTS

The study was supported by a grant from Rajavithi Hospital, Department of Medical Service, Ministry of Public Health of Thailand. The authors would like to thank all who participated in this study.

Disclosure

The author reports no conflicts of interest in this work.

REFERENCES

1. Moss A, Nalankilli K. Standardization of polypectomy technique. *Best Pract Res Clin Gastroenterol*, 2017; 31: 447-53.
2. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, Van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med.*, 2012; 366: 687-96.
3. Ferlitsch M, Moss A, Hassan C, Bhandari P, Dumonceau J-M, Paspatis G, et al. colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. *Endoscopy*, 2017; 49: 270-97.

4. Uraoka T, Fujii T, Saito Y, et al. Effectiveness of glycerol as a submucosal injection for EMR. *Gastrointest Endosc*, 2005; 61: 736-40.
5. Huai ZY, Xian WF, Jiang LC, Chen WX. Submucosal injection solution for endoscopic resection in gastrointestinal tract: a traditional and network meta-analysis. *Gastroenterol Res Pract.* 2015, Article ID 702768, 10 pages http://dx.doi.org/10.1155/2015/702768.
6. Hirasaki S, Kozu T, Yamamoto H, et al. Usefulness of 0.4% sodium hyaluronate solution as a submucosal fluid "cushion" for endoscopic resection of colorectal mucosal neoplasms: a prospective multi-center open-lebel trial. *BMC Gastroenterol*, 2009; 9: 1. doi:10.1186/1471-230X-9-1
7. Kishihara T, Chino A, Uragami N, et al. Usefulness of sodium hyaluronate solution in colorectal endoscopic mucosal resection. *Dig Endosc*, 2012; 24: 348-52.
8. Katsinelos P, Kountouras J, Paroutoglou G, et al. A comparative study of 50% dextrose and normal saline solution on their ability to create submucosal fluid cushions for endoscopic resection of sessile rectosigmoid polyps. *Gastrointest Endosc*, 2008; 68: 692-8.
9. Varadarajulu S, Tamhane A, Slaughter RL. Evaluation of dextrose 50% as a medium for injection-assisted polypectomy. *Endoscopy*, 2006; 38: 907-12.
10. Yandrapu H, Desai M, Siddique S, et al. Normal saline solution versus other viscous solutions for submucosal injection during endoscopic mucosal resection: a systematic review and meta-analysis. *Gastrointest Endosc*, 2017; 85: 693-9.
11. Conio M, Rajan E, Sorbi D, Norton I, Herman L, Filiberti R. Comparative performance in the porcine esophagus of different solutions used for submucosal injection. *Gastrointest Endosc*, 2002; 56: 513-6.
12. Polymerosa D, Kotsalidis G, Triantafylloua K, Karamanolisa G, Panagiotides JG, Ladasc SD. Comparative performance of novel solutions for submucosal injection in porcine stomachs: an ex-vivo study. *Dig Liver Dis.*, 2010; 42: 226-9.
13. Al-Taie OH, Bauer Y, Dietrich CG, Fischbach W. Efficacy of submucosal injection of different solutions inclusive blood components on mucosa elevation for endoscopic resection. *Clin Exp Gastroenterol*, 2012; 5: 43-8.
14. Bernard R. Fundamentals of biostatistics. 5th ed: *Duxbury Press*., 2000.