INTRODUCTION

Regenerative endodontic procedures (REP’s) can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex [1]. These procedures were put forward to overcome the drawbacks that are related to the clinical management of necrotic immature permanent teeth and are getting prominence over traditional apexification procedures amongst researchers and clinicians [2]. Recent studies revealed that the embryonic and adult stem cells of dental pulp have multipotency and angiogenic capacity that can be used therapeutically in dental pulp tissue engineering[1]. Regenerative procedures are not fully practiced by endodontists and remain an unresearched territory in Nepal. Though few case reports have been published but they are limited to revascularization via blood clotting. Regenerative endodontic procedures are slowly gaining acceptance in colleges in Nepal but till now no any survey has been conducted. Dental association and concerned body should take necessary step for establishing stem cell banks to collect dental tissue for regenerative purpose.

The knowledge and views of endodontists of various dental colleges and clinics help to know the present status of these procedures in their practice and bolster any updates needed for practicing these procedures safely and routinely. This will also be beneficial in determining any modifications needed on REPs in the current postgraduate curriculum. Thus, the objective of this study was to investigate the learning, perception and practice among
endodontists towards regenerative endodontics and factors affecting their insight, demeanour, and practice.

MATERIALS AND METHODS
Study design and setting
It was a cross-sectional questionnaire study conducted during the month of Jan 2022 to April 2022 among Nepalese Endodontists through electronic communication media (Viber, Messenger and WhatsApp) to assess their knowledge, attitude and practice of regenerative endodontics in dentistry.

Participants and procedure
Google form consisted the questions was created and the link was distributed amongst the Endodontists of Nepal. The link containing the Google form was sent personally to almost every Endodontists through electronic communication media (Viber, Messenger and WhatsApp) and their responses were recorded. Besides this, it was also posted on online closed viber group ‘Endodontists of Nepal’. The list of Nepalese Endodontists working in various regions of Nepal was obtained from CDEAN (Conservative dentistry and Endodontics Association of Nepal) and Viber group, ‘Endodontist of Nepal’ where most of the Endodontists are connected.

Total 20 multiple choice questions were generated in this study on the basis of previous similar studies,[1-3,6,7] which was used for a pilot study among 10 endodontists and was later modified after discussion with three endodontist colleagues. The data from pilot study wasn’t included in the study. The questionnaire consisted of four parts. The first part contained questions regarding profile of respondents including demographics and years of experience. The second part contained 7 questions that were framed to evaluate the knowledge, third part contained 6 questions to evaluate the attitude, and fourth part contained 7 questions to disclose practice regarding regenerative endodontics in clinical scenario. First part was required mandatory to be filled by participants or response cannot be recorded. The questionnaire was in English language. Responses to the questionnaire were accepted for a period of 4 months.

Statistical analysis and data management
All the collected data was filled in Excel sheet and it was transferred into Statistical Package for Social Sciences (SPSS) (version 21.0) for statistical analysis. Descriptive statistical analysis was performed. Frequency and percentage were calculated. Pie chart and simple bar diagram were presented.

Ethical considerations
This study was approved by Institutional Review Committee (IRC No: 2079/80/72) of KIST Medical College Teaching Hospital. All information was kept confidential; anonymity was emphasized and maintained. The personal identifier was removed at the time of data analysis and presentation.

RESULTS
There were 65 respondents, out of which 62.5% (n=40) were females and 37.5% (n=24) males with a female to male ratio of 1.66. Majority (67.7%, n=44) of the participants were below 40 years of age and participants above 40 years were 32.3% (n=21). Most (72.3%, n=47) of the participants were in practice for more than 5 years.

All 100% (n=65) of the participants have come across the term regenerative endodontics and the source of information is mainly through post graduate training (63.1%, n=41). Majority (71.9%, n=46) of participants are in view that regenerative endodontics is a broad term encompassing apexogenesis, pulp revascularization, pulp implantation and three-dimensional cell printing. Respondents had good knowledge about the sources of dental stem cells (87.3%, n=55). Most respondents were well known about the type of stem cells found in human exfoliated deciduous teeth while few (8.1%, n=5) respondents were unknown about it (Figure 1).

The majority (87.3%, n=55) of respondents were aware of the fact that leaching of sodium hypochlorite into periapical area has a detrimental effect on revascularization procedure. Likewise, 25.6% (n=15) of the respondents were versed with the knowledge to recommend Ethylene-Diamine-Tetra-acetic acid (EDTA) during revitalization procedure. Majority (59.7%, n=37) of the respondents considered lack of adequate knowledge as a barrier in regenerative treatment.
while 8.1% (n=5) looked regenerative endodontics as complicated procedure. Most (53.2%, n=33) of them had received continuing education about application of regenerative endodontic procedures while 46.8%(n=29) had no any experience of such training. A high percentage (98.4%, n=62) of respondents strongly recommended regenerative therapy to be incorporated into dentistry and dental professional associations should also regulate the use of stem cells and regenerative dentistry (82.5%, n=52). Similarly, 52.4% (n=33) of respondents had a perspective that revascularization procedure is more successful on teeth with a large apical foramen than small apical foramen. Likewise, 54% (n=34) of respondents were in strong belief that healing of peri-radicular bone, continued root development in immature teeth, pulp tissue revitalization and tooth reimplantation are equally valuable type of REP’s.

A good percentage (82.5%, n=52) of respondents practiced REPs with successful treatment outcome of 80% (n=44). Most (40.3%, n=25) of the participants in their daily clinical practice dealt with 26-50% of teeth with peri-radicular lesions while 66.1% (n=41) of respondents dealt with less than 10% of necrotic immature teeth. Among the participants of this study, 54.1% (n=33) considered triple antibiotic paste and pulpal regeneration to be the optimal treatment of such necrotic immature teeth (Figure 2). Similarly, 83% (n=44) of respondents introduced stem cells into the canal by inducing bleeding from the periapical region. A high percentage (87.1%, n=54) of respondents had a belief that regenerative endodontic treatment could serve as the alternative treatment option to dental implant.

The most accepted treatment option for teeth with infected or non-infected necrotic pulp is root canal treatment where the root canal space is disinfected and filled with biocompatible material to prevent reinfection of the canal space for decades. It was expected that the root canal filling would prevent coronal leakage, retard bacterial penetration from the canal space into the periapical tissues, and probably entomb bacteria within the canal space only. But it is unfortunate to say that root canal filling is unable to achieve these desirable expectations in all endodontically treated teeth.

DISCUSSION

The most accepted treatment option for teeth with infected or non-infected necrotic pulp is root canal treatment where the root canal space is disinfected and filled with biocompatible material to prevent reinfection of the canal space for decades. It was expected that the root canal filling would prevent coronal leakage, retard bacterial penetration from the canal space into the periapical tissues, and probably entomb bacteria within the canal space only. But it is unfortunate to say that root canal filling is unable to achieve these desirable expectations in all endodontically treated teeth.

From several decades, Endodontists have been looking for biologically based procedures with the aim to replace damaged dentin and root structures as well as cells of pulp-dentin complex that has been destroyed by infection or trauma [2,8]. Regenerative endodontics is a step ahead of traditional root canal therapy and more aggressive treatments such as surgical endodontics in a sense that it preserves the vitality of tooth thus extending its life span [2].

It seems that the Endodontists and Pedodontists would be the first practitioners to deal with such treatments. Pedodontists frequently deal with deciduous teeth where regenerative endodontic procedures are contraindicated [9]. So, the endodontists are at the forefront of this cutting-
edge research. The knowledge and attitude of these practitioners play a pivotal role in the success of any regenerative endodontic procedure. Hence, this study is aimed at collecting the level of knowledge, attitude and practice of Endodontists towards REP’s. To the best of our knowledge, this is probably the first survey in the field of regenerative endodontics in Nepal.

In this study, all the respondents (100%) were highly aware of the term ‘Regenerative Endodontics’ showing their greatest interest in this emerging field. Similar studies were conducted in other parts of world in the past, but the level of awareness of the participants was not as good as in this study. In a study conducted amongst dental professionals of Manguluru, 97% of the respondents had come across the term ‘Regenerative Endodontics’[2]. Likewise, in a similar study conducted amongst dental residents in selected hospitals within Nigeria, 91.2% of the participants had come across the term regenerative endodontics [10]. High awareness of the respondents in this study could be attributed to the increase in understanding about this topic mostly through post graduate training (63.1%, n=41). A small percentage (4.6%, n=3) had attended conference, symposium or seminar. Most of them (53.2%, n=33) had never received continued education about application of regenerative endodontic procedures which suggests that this topic has not got place in mass discussions among clinicians. This is why majority (82.5%, n=52) of the respondents were in a view that dental professional associations should regulate the use of stem cells in regenerative endodontic procedures and it should be strongly incorporated into dentistry (98.4%, n=62). In a cross sectional survey conducted amongst Endodontists from four universities in India, 86.5% (n=83) thought that regenerative therapy should be incorporated into dentistry and 79.2% (n=76) believed that dental professional associations should come forward for the upliftment of using stem cells and regenerative endodontics [6].

Majority (71.9%, n=46) of the respondents in this study were aware of the fact that regenerative endodontics is a vast topic with pulp revascularization, apexogenesis, pulp implantation and three-dimensional cell printing under its umbrella. A differentiation between endodontic repair, regeneration and revascularization must be understood before utilizing them in clinical practice. Repair indicates that healing of damaged tissues occur by remaining vital tissues, where the original odontoblasts survive restoring the pulp tissue to normal like form and function. Regeneration indicates an objective of reproducing original pulp like tissue both histologically and functionally where the original pulp tissue is completely necrosed [11]. Revascularization induces thickening of the canal walls radiographically by the deposition of hard tissues and continued root development in immature permanent teeth with necrotic pulp and apical periodontitis or abscess [12].

Respondents in this study had fair knowledge about the dental stem cells (59.7%, n=37) and their sources (87.3%, n=55). A significant number of dental mesenchymal stem cells (MSCs) have been found in teeth and their supporting structures. After the discovery of dental pulp stem cells (DPSCs) from human impacted third molars by Gronthos et al, stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicle precursor cells (DFPCs), stem cells from apical papilla (SCAP), and gingiva-derived mesenchymal stem cells (GMSCs) have been subsequently identified [13]. The essential triad of tissue regeneration are stem cells, growth factors and a scaffold [8]. In this study, 82.5% (n=52) of the respondents were using membranes, scaffolds or bioactive materials in their regenerative endodontic practice which is higher than the findings reported by Assiry A A et al [9]. This suggests that many regenerative endodontic procedures are already in common use. In regenerative endodontic procedures of immature permanent teeth with necrotic pulps, periapical bleeding is induced so as to bring mesenchymal stem cells, fibrin scaffold and blood-derived bioactive growth factors from periapical area into the canal space. In this study, the most recognized method of introducing stem cells into the canal was by inducing bleeding from periapical region (83%, n=44) which is similar to the study conducted by Ariwala F et al. where maximum participants (59.2%) follow the same technique [2]. Disinfection of the root canal space is of utmost importance in the success of any regenerative endodontic procedure for which both sodium hypochlorite and...
Ethylenediaminetetraacetic acid (EDTA) irrigants can be used [2]. Majority of the respondents (57.4%, n=35) recommended both the irrigants during regenerative endodontic procedure. This awareness is greater than the awareness of respondents of study conducted by Ariwala et al (35.9%, n=161) [2]. But extrusion of sodium hypochlorite into periapical tissues was considered detrimental by majority of respondents (87.3, n=55). Higher concentration of sodium hypochlorite has adverse effect on the survival and differentiation of stem cells of the apical papilla (SCAP). This adverse effect can be prevented by using lower concentration (1.5%) of sodium hypochlorite followed by 17% EDTA [4,14]. Irrigation of the canal space with EDTA demineralizes dentin causing release of growth factors embedded in the dentin matrix [8]. Along with this it promotes the adhesion, migration and differentiation of dental pulp stem cells onto dentin [4].

Preoperative apical diameter of the tooth has greater significance in regenerative endodontic procedures. Teeth with apical foramen larger than 1 mm are likely to show a greater increase in root thickness, length and apical narrowing. This finding suggests that there might be a need of instrumentation of fully formed (closed) apices of tooth with necrotic pulp to approximately 1 to 2 mm during revascularization to allow systemic bleeding into root canal systems [15]. In this study, 52.4% (n=33) of the respondents also supported the view that revascularization procedures on teeth with a large apical foramen are more successful than teeth with small apical foramen.

The majority (54.1%, n=33) of the respondents considered triple antibiotic paste and pulpal regeneration as the ideal treatment option for necrotic immature teeth. This is in agreement with a study conducted by Assiry A A et al [9]. Most of the respondents (66%, n=41) in this study practiced necrotic immature teeth less than 10% in daily clinical practice. Meanwhile 40.3% (n=25) of the respondents came across 26-50% of teeth with periradicular lesions. A study conducted by M Deborah A et al reported 60.7% of respondents dealt with less than 10% of necrotic immature teeth and 31.5% came across 26-50% of teeth with periradicular lesions in their daily practice [10]. This finding discloses the fact that there is lack of cases for Endodontists where REPs can be incorporated as a standard treatment option in endodontics. Lack of sufficient knowledge about stem cells in dental practitioners was much a greater concern in practicing regenerative endodontic procedures as stated by 59.7% (n=37) of the respondents. This clearly emphasizes the need for continued training and continuing education programs that focuses on the incorporation of stem cell therapy in endodontics. In a similar study conducted by Manguno C et al, higher cost was the biggest obstacle to a patient accepting regenerative endodontic treatment [16]. More than 50% of the respondents were congruent with the view that healing of periapical lesion, continued root development in immature teeth, pulp tissue revitalization and avulsed tooth reimplantation all fall under regenerative endodontics with equal value. Bakhtiar et al. in their case series reported the healing of periapical lesions with root developments and apical closure in all cases when platelet rich fibrin was used as a scaffold in immature necrotic teeth [6].

Here, 87.1% (n=54) of respondents thought that regenerative endodontic procedure could serve as the alternative treatment option to dental implant. This data coincides with the data reported by Manguno et al [16]. and is higher than the data reported by Epelman I et al [17].Most of the study participants in this study were optimistic about regenerative endodontics. They were already practicing some kind of regenerative endodontic procedures with successful outcome (80%, n=44). In a study conducted by Manguno C et al., only 35.5% (n=11) reported the outcome of their regenerative dental treatment as successful [16]. But there are literatures suggesting that REPs are not always successful. There are some potential complications and undesired outcomes like tooth discoloration, adverse reaction to intracanal antibiotics and treatment failure [18]. Along with this intracanal calcifications in form of either calcific barrier or canal obliteration have been reported in endodontic cases after revascularization [19]. These should be discussed with the patients and guardians before start of the treatment. Though regenerative endodontics is one of the most fascinating developments in dentistry today, it is equally challenging. Control of microbes as in conventional
root canal treatment and spatial control of release of growth factors from the scaffold remain as a major challenges [20].

CONCLUSIONS
Regenerative endodontics is a vast field where more research is still needed so as to reveal many unknown facts and explore new techniques and technologies. Within the limitation of the study which included focusing only on endodontists rather than a larger pool of dentists it can be concluded that the Endodontists had sound knowledge and positive attitude towards regenerative endodontics but a need for regular practice was felt. More surveys like this should be conducted in the future to increase in understanding and awareness on this topic.

ADDITIONAL INFORMATION AND DECLARATIONS
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