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THE USE OF HUMAN PLACENTAL EXTRACT TO ARREST AND RESTRICT THE ESTABLISHED CARIOUS LESIONS WITH THE AID OF NANO FIBERED LOCAL DRUG DELIVERY SYSTEMS: A TWO YEARS' EVIDENCE BASED STUDY

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ABSTRACT

The use of human placental extract as a therapeutic agent has caused revolution in the medical field .This is due to its immunomodulatory effect, numerous growth factors and powerful regenerative property. In this study we made use of these unique properties of the human placental extract in order to cause arrest of the established carious lesions. A treatment aiming at tissue healing, regeneration and thus dentinogenesis. Local drug delivery systems were used to aid in sustained, controlled and targeted release of the placental extract .Eighty patients were included in this study. The patients were divided into two groups of 40. Group (P) received the placental extract treatment and was further divided into two sub groups of 20 (Pa&Pb) according to the method of administration of the placental extract and the pharmaceutical modulation of the release and targeting process. The other group (C) was a control group and did not receive the placental extract treatment. After two weeks all patients were investigated clinically for signs and symptoms and radiographically for pathologic radiographic changes. Follow up of all patients was carried out over three and six months and two years time. Regarding group (P), sub group (Pa), except for one case, at all time periods, nineteen cases demonstrated appropriate function and normal periodontium apparatus and total absence of any clinical signs and symptoms .The same went with sub group (Pb) except for two cases. Regarding the control group (C), at all time periods, twenty one cases did not show any clinical signs symptoms and demonstrated normal periodontium apparatus. Five cases failed over six months and needed retreatment. Nine cases failed in two years and needed retreatment. While five cases failed in two years and needed traditional endodontic treatment.

KEY WORDS: Human placental extract, caries, regeneration, local drug delivery systems.

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INTRODUCTION

Dental caries has always been described as one of the most prevalent conditions worldwide[1]; which accounts for significant morbidity[2]. A direct effect of untreated dental caries on oral health and associated quality of life has been reported .Nevertheless, identification of associations between dental caries and systemic health, though of potential interest, have gain little attention [3].The oral cavity is the connection between medicine and dentistry and an indication of the general health [4].It has been reported that about one hundred systemic diseases and five hundred medications have oral manifestations[4]. There are multiple types of surfaces in the oral cavity .Each is colonized by a unique population of more than 500 -700 species of viruses, bacteria, fungi and protozoa .Most of these species are significantly virulent[5-9]. Oral diseases impact systemic health and vice versa [10]. There are bidirectional relationships between oral and systemic conditions that are being better realized to date[4]. There are multimodal relationships and factors that connect dental diseases to systemic diseases and conditions [10].The breakdown of oral and dental tissues, in various cases, is the result of a direct mechanism involved in severe acute systemic disorders. This may act as an allostatic load or overload paradigm of chronic stress contributing to rapid breakdown and loss of tissues by various mechanisms .Thus the allostatic load leads to wear and tear on the human body as a result of chronic stress . And this, in turn, contributes to pathology[10].

In dental caries, involvement of the pulp, root canal space or the periodontium are the likely pathways for direct systemic invasion of oral microbiota[11]. Host factors and pathogenic traits can promote dental caries and increase the likelihood of systemic spread .These factors include diseases, medications and adhesion expression in streptococcus mutans for collagen binding[12,13]. The immune-pathological role and pattern largely affects and connects dental and systemic diseases resulting in a bidirectional relationship between oral and systemic health (Figure 1). The recent studies reported that stimulation of the circumpulpal odondoblasts followed by release of cytokines and recruitment of immunological cells occur in the early stages of dental caries [11].

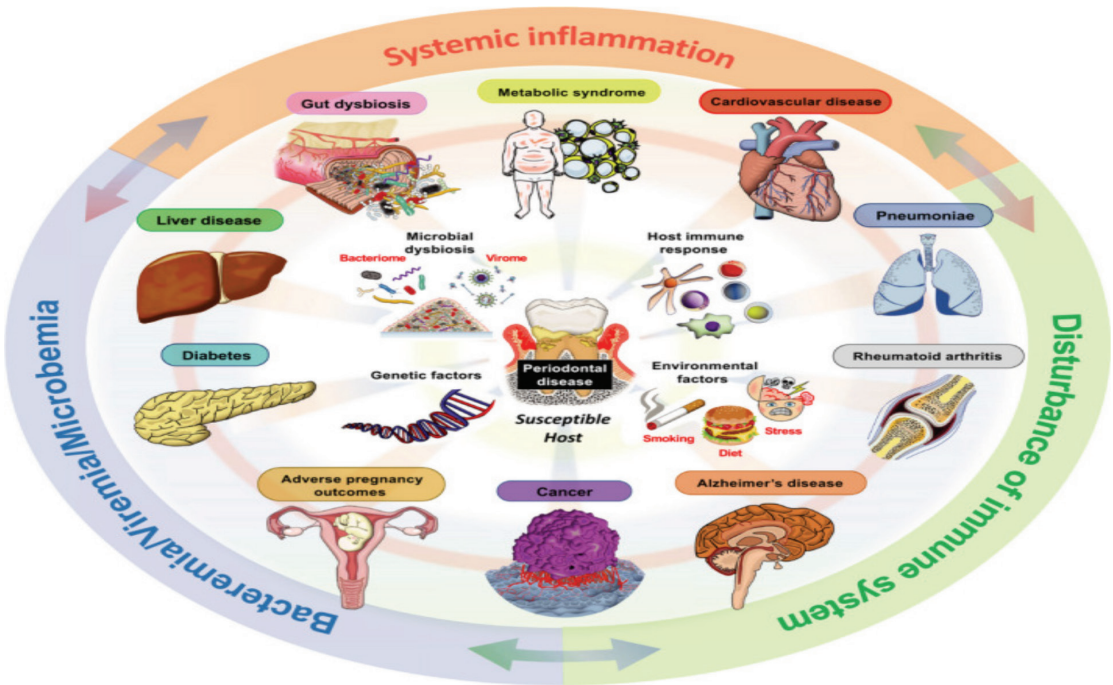

Fig. (1) Dental disease associations with systemic diseases and conditions [10]

The immunological pattern in caries involves the activation of the nuclear factor kappa B (NF-KB), followed by biosynthesis of pro-inflammatory cytokines and release of T-lymphocyte helper (Th)-1. The unrestricted disease progression results in activation of Th -17 response mediated by M-1 macrophages [11]. This shared immunological pattern induces synergistic pro-inflammatory effects among dental and systemic diseases [11].The placenta is now described as a substance that will revolutionize modern medicine[14-16]. Currently, the human placental extract is approved for clinical use [17-20]. It has also been reported as safe with no significant side effects and contraindications[17-20]. A thorough understanding of the immune reactions of dental tissues, of cellular and molecular key players, as well as the patterns of defense will indicate a treatment that is less invasive and aimed at tissue healing and regeneration rather than replacement [21,22]. In light of the immuno- pathological status mentioned above, odontoblasts, due to their anatomic position at the periphery of the pulp, form the first line of defense. With pathogen recognition, odontoblasts and immune cells (found in the pulp), induce a multitude of signaling molecules to control the immune response. Initially the odontoblasts release chemokines (CCL2, CXCL1,CXCL2,CXCL8 & CXCL10)followed by the release of various cytokines (IL_1a, IL_1b, IL4, IL6, IL8 &IL10) which are known to control different aspects of the inflammatory response according to their levels and profiles. Also nitric oxide (NO), synthesized by the enzyme (NO) syntheses plays a large role in these immune reactions[22]. The placental extract has been reported to have strong immunomodulatory properties .It produces (NO) which plays a large role in these immune reactions. Also the placental mesenchymal stem cells (PMSCs) prevent proliferation and release of cytokines by T helper -1 cells and, in the mean time induce the expression and secretion of T helper -2 cells.

Moreover, the (PMSCs) induce the differentiation of the T regulatory lymphocytes and Th-2 polarization (accompanied with increased levels of IL-4 and IL-10). This is performed by direct (PMSCs) contact with T-cells. Therefore the (PMSCs) serve to attenuate T-cell activity [23]. In addition to immune regulation, the placenta offers hormonal and nervous regulation as well. Moreover, the placenta possesses various physiologically powerful growth factors, the most important of which are: Hepatocyte growth factor (HGF) promoting growth of liver paranchymal cells and various other tissues. Nerve growth factor (NGF), that promotes growth of nerve cells (sensory and sympathetic). Epidermal growth factor (EGF), that promotes growth of skin, cornea, lungs and tracheal epithelial cells .Fibroblast growth factor (FGF) that promotes growth of fibroblasts and vascular endothelial cells .Insulin -like growth factor (IGF),that promotes growth of cartilage cells and smooth muscle cells . Growth factors that elevate immune strength. Colony-Stimulating factor (CSF), that promotes growth of stem cells . Interleukin-1(IL-1), that promotes production of immune-competent cells (T- cells, B- cells, and NK - cells), thymus cells and lynphokines. Interleukin -2 (IL -2), that promotes growth of T- cells (helper, killer and suppressor T-cells).Interleukin-3 (IL-3),that promotes growth of hematopoietic cells and mast cells .Interleukin -4(IL-4), that promotes growth of B cells as well as division of antibody - producing cells. Amazingly, the hepatocyte growth factor (HGF), has been reported to have regenerative effect on cells and organs. Other powerful ingredients of the placenta include: DNA, RNA and metabolic products; essential amino acids as Leucine, Glycine,Valine and Threonine; active peptides; certain vitamins (B1, B2, B6, B12, C,D,E& niacin); certain minerals (Ca, Na, K, P, Mg, Zn &Fe);enzymes (over 100 enzymes including ALK phosphatase, acid phosphatase & ADB)and saccharides. So, it can be deduced that the placenta (clinically and medically) demonstrates strong immunomodulatory properties resulting in appropriate immune system regulation as well as autonomous nervous system control, endocrine

hormone control, liver function enhancement, attenuation of basal metabolism, improvement of physical constitution, anti-inflammatory action, improvement of blood circulation, treatment of anemia and wound healing [17-24]. Also, the human placental extract is clinically used for skin regeneration in treating skin wrinkles[25]. Moreover, the human placental extract is included in various skin ointments that promote skin revitalization and melanocyte growth as well as for treatment of dermatitis and psoriasis [21].Research on stem cells offers advanced knowledge about development of an organism from a single cell, and how healthy cells replace damaged ones in adult organisms .Stem cells (also found in the placenta) have the ability to continuously divide to either replicate themselves (self-replication),or to produce specialized cells that can differentiate into other types of cells and tissues (multilineage differentiation)[21,22].Due to the powerful regenerative effect of the placenta, its strong immunomodulatory properties, its possession of growth factor, enzymes, vitamins and minerals; a treatment aiming at tissue healing and regeneration and thus dentinogenesis can be carried out. Subcutaneous or intra muscular injection of human placental extract which is a hydrolysate of the human placenta commercially named Laennec (Japan Bio Products, Tokyo, Japan)has recently been used for treatment of various oral, dental and systemic disorders.[17-25]

MATERIALS AND METHODS

This study was ethically approved of by the ethical committee of the faculty of dentistry –Pharos University-Egypt and with an ethical approval number PUA02202002233010

Selection and grouping of subjects

A total of 80 patients were included in this study. They were divided into two groups of 40.The first group (P) was to undergo treatment using human placental extract(HPE) (table1),while the second group(C) was the control group. All patients aged 25-55 years old. Group (P) was further divided into two subgroups of 20, (Pa/Pb) according to the method of administration of the placental extract. For all patients confirmation was made to ensure there were no medical contraindications for dental treatment. Moreover, routine blood investigations as complete blood count (CBC),RBS, HIV,and HBSAg were performed before the beginning of the treatment. Also by thorough clinical examination, all patients had good oral hygiene. Posterior first and second molars were selected for this study. For all patients, teeth were examined to ensure pulp vitality. Also it was confirmed that the patients were not allergic to medicaments and antibiotics necessary for the clinical procedure of the study. All patients demonstrated moderate to severe degrees of carious lesions (Si1 Sta2, Si2 Sta2, Si1 Sta3, and Si2 Sta3) i.e. the carious lesions involved the middle third of the dentinal substrate and extended deeper, as revealed by the bitewing radiographs. Before initiation of the treatment an informed consent from each patient included in the study was obtained. The consent form included the number of appointments, the use of medications and antibiotics and their possible adverse effects such as (infection, pain, and/or lack of response to treatment); and the possibility of resolving to traditional endodontics or even extraction in situations beyond salvage. The patients included in this study were family members, friends and business associates so as to facilitate recall and follow up through the study period. Safety of the human placental extract product LAENNEC (table 1, figure 2), according to the manufacturer:

- The donor of placenta is medically checked. She should be free from tuberculosis, syphilis, gonorrhea, HBV, HCV, HIV, HTLV [adult T cell leukemia], HPV /B19.
- She should have been in Japanese hospitals only, to ensure certain strict standards.
- LAENNEC safety is confirmed by the most strict safety measures among existing scientific standards.

- The manufacturing process is performed by three different proprietary techniques.
- In order to, further, improve safety, the sterilization process is performed several times during the manufacturing process to eliminate any possibility of contamination;

1. The placenta is hydrolysed by hydrochloric acid and heat treated for 15-17 hours.
2. During the final process, it is sterilized by steam at 121 degrees (Celsius) for 30 minutes.

- By those two last processes all viruses get killed, and prions get destroyed.
- Laennec is the only placental extract free from hormones and steroids.
- No serious side effects were reported.

TABLE (1) Materials, components, batch numbers and manufacturers

Material	Components	Batch numbers	Manufacturer
LAENNEC	Human placental extract	30472	Japan Bio Products YHB PharmaTokio/Japan
3M™ESPE™ Adper™ Single Bond2	Dental adhesive system Phosphoric acid etchant	NA93543	3MESPE Dental product St Paul, MN, USA
3M Filtek™ Z250XT	Nanohybridcomposite Inorganic filler:Zirconia/Silica(60%by volume Matrix: BIS-GMA,UDMA and BIS-EMA resins	NF40669	3MESPE Dental product St Paul, MN, USA
Orafil-G®	Temporary filling material	PK2122243	PrevestDenPro®
Chitosan	Poly(D-glucosamine)deacetylated chitin MW.100-300KDa	wwETU665rg	LANxess company, India
Polylactic –co-glycolic acid	Poly(D,L-lactic-co-glycolide)Lactide-glycolide50:50 MW45000 g/mol	MKCP6015	SIGMA –ALDRICH, USA
Hydroxyl propyl methyl cellulose	Hydroxyl propyl methyl cellulose	131212498	Biopharm, Egypt

Fig. (1) Placental extract used in this study

Clinical procedure

At the first appointment and for each patient of both groups, anaesthesia was performed and the tooth isolated using a rubber dam. Caries was then removed using high speed burs and a cavity was performed in accordance to the general principles of cavity preparation. Si1/Sta2, Si1/Sta3, Si2/Sta2 and Si2/Sta3 cavity preparations were performed according to the extent of caries invasion. That was followed by copious rinsing. The placental extract was used with the aid of a local drug delivery system (LDDS) (table 1) in order to; transfer the loaded placental extract to the target site (the dental pulp)

and provide a sustained and controlled release of the placental extract. That, in turn, would increase the efficacy and local concentration of the placental extract. The local drug delivery system included three types of polymers; chitosan, polylactic–co-glycolic acid and hydroxy propyl methyl cellulose. Chitosan powder (4%) was dissolved in acetic acid solvent (of concentration 90%) by the aid of a magnetic stirrer at a temperature 50Cº to increase solubility ending up by a pale yellow clear homogeneous gel. Polylactic –co- glycolic acid [16%] as powder was dissolved in chloroform solvent using the magnetic stirrer and ending up by a yellow clear homogeneous gel. Hydroxy propyl methyl cellulose (60%) as powder was dissolved in deionized water and also using the magnetic stirrer to end up by a clear white homogeneous gel. The hydroxy propyl methyl cellulose polymer was electrospun in a nano-electrospinning device (NANON-01B, serial no. 13721 LO11, MECC co, LTD. Japan) (figure 3). Afterwards, two sets of separate syringe pumps and power supplies together with a common collector were used to produce simultaneous induction of chitosan and polylactic-co-glycolic acid polymers into the nano-electrospinning device, and,also, were thoroughly electrospun. The spinning rate was 0.1ml / hr. at a voltage of 28kV.The purpose of electrospinning is to convert the polymers into Nano fibered structures Also, during the electrospinning process, all the polymers solvents got evaporated. The generated electrospunnanofibers were deposited and pumped via the collector of the nano-electrospinning device, then collected on an aluminium surface. The collected generated electrospun nanofibers were, then, twisted to produce a bundle of nanoyarn (figure 4).The electrospinning process resulted in a lower controllable viscosity of the mix, total evaporation of polymers solvents and, more important, generation and production of nanofibers in order to facilitate a sustained and controlled release of the placental extract through dentinal tubules as well as targeting of the placental extract to the dental pulp.

Fig. (1) The Nano-electrospining device used in this study

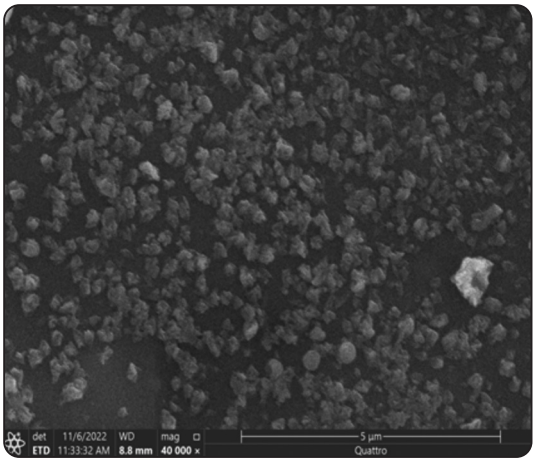

Fig. (2) SEM showing the Nano fibers structure of electrospun polymers

Regarding the subgroup (Pa), the resultant electrospun bundle of nanofibers (nanoyarn), was manually twisted and mixed with the placental extract; then the resultant was cut into pieces to suit the prepared cavities. Each piece was inserted and adapted in an individual prepared cavity overlying the pulpal floor(none of the selected teeth showed frank pulp exposure) then covered by a temporary restoration. Regarding, the subgroup (Pb), the same electrospinning process was carried out but, unlike, the subgroup (Pa), the placental extract was not added or mixed, primarily, with the resultant electrospun bundle of nanofibers (nanoyarn). Instead, the resultant nanoyarn was cut into pieces and soaked in the placental extract

for 10 min. Afterwards, each piece was placed and adapted to the cavity pulpal floor and covered by a temporary restoration as explained before. The patients were dismissed and recalled in two weeks time for a second appointment. During the second appointment, all patients were meticulously clinically examined; for signs and symptoms of persistent infection as swelling, tenderness, pain, tooth mobility and fistula; and radiographically; to monitor any alteration of lamina dura, widening of the periodontal ligament, any signs of pathologic external or internal root resorption and any interradicular or periapical radiolucency.With the absence of the above mentioned clinical and radiographic signs and with the appearance of a dentin bridge, radiographically, (for group P), each patient,(in both groups), was anesthesized and a dental rubber dam was used for isolation. All cavity walls were, then, conditioned and bonding performed using Single Bond(3M ESPE), according to the manufacturers instructions, (table1). The restoration was, then, performed using the resin composite restorative material Filtek Z 250 XT (3M ESPE), (table 1). All treated teeth, for both groups, were re-evaluated after three and six months and two years, for clinical signs and symptoms as; swelling, pain, tenderness, tooth mobility and fistula; using percussion tests as well as palpation of teeth and alveolar areas. Also, radiographic investigations were performed to assess any changes as, loss of lamina dura, widening of the periodontal ligament, any signs of pathologic internal or external root resorption, and any interradicular or periapical radiolucency.

RESULTS

The grade of success is attained by achieving the following targets:

- Absence of clinical symptoms as; swelling, pain, tenderness, tooth mobility and fistula.
- Absence of undesirable radiographic examination results as: loss of lamina dura, widening of the periodontal ligament, any signs of pathologic internal or external root resorption and any periapical or interradicular radiolucency.
- Presence of a clear dentin bridge.
- Positive response to vitality testing.

Clinical examination

Regarding group (P); for the subgroup (Pa), at the second treatment appointment, (after two weeks), and at all evaluation time periods, (three and six months and two years), nineteen of the treated teeth demonstrated appropriate function as well as absence of clinical signs and symptoms as pain, tenderness, swelling, tooth mobility and fistula. One case failed in two years time and needed retreatment. Vitality tests showed all twenty cases to be vital. For the subgroup (Pb); at the second treatment appointment, (after two weeks), and at all evaluation time periods, (three and six months and two years), eighteen of the treated teeth demonstrated appropriate function and absence of clinical signs and symptoms. Two cases failed in two years time and needed retreatment. Vitality tests showed all twenty cases to be vital. Regarding group (C); at the second treatment appointment, (after two weeks), and at all evaluation time periods,(three and six months and two years), twenty one of the treated teeth showed absence of clinical signs and symptoms as pain, swelling, tenderness, tooth mobility and fistula. Five cases failed in six months period and needed retreatment. Nine cases failed over two years time and needed retreatment. Meanwhile, five cases failed over two years time and needed traditional endodontic treatment. Except for the cases that needed endodontic treatment; vitality tests showed all other cases to be vital. Radiographic examination. Regarding group (P); at the second treatment appointment (after two weeks), and at all evaluation time periods (three and six months and two years); all cases belonging to both sub groups(Pa and Pb), showed appropriate function with normal periapical tissues and normal periodontium. Also, the cases did not demonstrate any loss of lamina dura, widening of the periodontal ligament, signs of pathologic internal or external root resorption, or periapical or

interradicular radiolucency. Moreover, the nineteen successful cases belonging to the subgroup (Pa), in addition to the eighteen successful cases belonging to the subgroup (Pb); all demonstrated clear dentin bridges. Regarding group (C); at the second treatment appointment (after two weeks), and at all evaluation time periods (three and six months and two years); the twenty one cases that did not fail appeared in function with normal periapical tissues and periodontium. Also, there was no loss of lamina dura, widening of the periodontal ligament, signs of pathologic internal or external root resorption, or periapical or interradicular radiolucency. The five cases that failed in six months time and the nine cases that failed over two years time, all demonstrated changes in the laminadura. Meanwhile the five cases that failed in two years time and needed traditional endodontic treatment; demonstrated radiographic changes indicating failure as; loss of lamina dura, widening of the periodontal ligament and, sometimes, periapical radiolucency.

Statistical Analysis

Data analysis was performed in several steps. Chi square test was used to compare between groups. Statistical analysis was performed using Graph-Pad Instat statistical software for windows. P values ≤0.05 are considered to be statistically significant in all tests.

The results of the clinical success/failure rate after two years of follow up were collected, tabulated and graphically drawn as follow;

In group C, after 3 months hundred percent (100%) of the examined subjects scored "success" with no "failure". After 6 months (87.5%) of the examined subjects scored "success" while (12.5%) scored "failure". After 2 years (60%) of the examined subjects scored "success" while (40%) scored "failure". (table2, figure 5).

In Sub-group-Pa, after 3 and 6 months hundred percent (100%) of the examined subjects scored "success" with no "failure". After 2 years (95%) of the examined subjects scored "success" while (5%) scored "failure". (table2, figure 6).

In Sub-group-Pb, after 3 and 6 months hundred percent (100%) of the examined subjects scored "success" with no "failure". After 2 years (90%) of the examined subjects scored "success" while (10%) scored "failure". (table2, figure 7).

The difference in clinical success/failure rate scores after two years of follow up between both groups was significant as indicated by chi square test ($p=0.0001<0.05$)

The difference in clinical success/failure rate scores after two years of follow up between both Sub-group_Pa and Sub-group_Pb was non-significant as indicated by chi square test ($p=0.4042 > 0.05$)

The difference in clinical success/failure rate scores as function of follow up periods was significant as indicated by chi square test ($p=0.0001<0.05$)

TABLE (2) Distribution of clinical success/failure rate scores after two years of follow up for both groups

Variables		***Gr_C***		***Gr_P***			
				Subgr_Pa		***Subg_Pb***	
		Success	***Failure***	***Success***	***Failure***	***Success***	***Failure***
Evaluation time	***3M***	40 (100%)	0 (0%)	20(100%)	0 (0%)	20(100%)	0 (0%)
	6M	35 (87.5%)	5 (12.5%)	20(100%)	0 (0%)	20(100%)	0 (0%)
	2Y	21 (60%)	14 (40%)	19 (95%)	1 (5%)	18(90%)	2 (10%)
Chi test	***P value***	0.0001*					

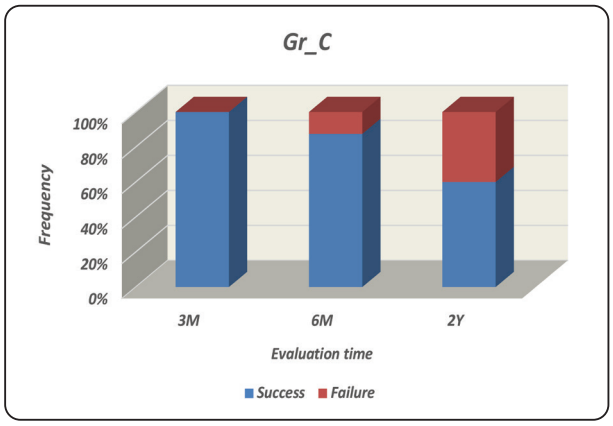

Fig. (5) Stacked column chart showing frequent distribution (%) of clinical success/failure rate scores after two years of follow up for Group **C**

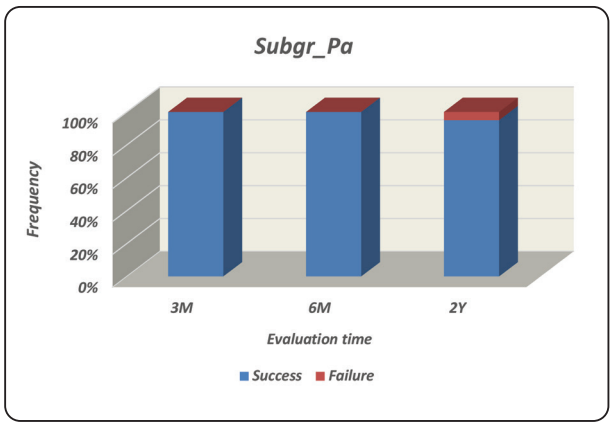

Fig. (6) Stacked column chart showing frequent distribution (%) of clinical success/failure rate scores after two years of follow up for **Sub-group_Pa**

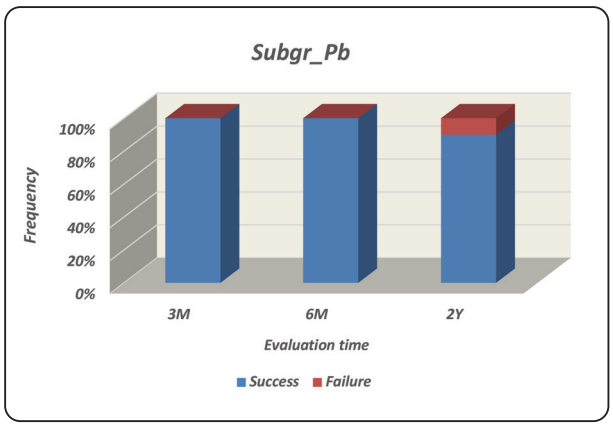

Fig. (7) Stacked column chart showing frequent distribution (%) of clinical success/failure rate scores after two years of follow up for **Sub-group_Pb**

DISCUSSION

Recently, it has been proven that dental caries progression involves a certain immunological pattern that results in activation of the nuclear factor Kappa B, followed by biosynthesis of pro-inflammatory cytokines and release of T- lymphocyte helper cells. Stimulation of the circumpulpal odontoblasts with the release of cytokines and recruitment of immunological cells, occur in the early stages of dental caries[10-13]. The placenta has, recently, been described as a therapeutic agent that caused a strong revolution in medicine[14-16]. The placental extract has been reported to possess strong immunomodulatory properties, thus playing an important role in immune reactions[22, 23]. The placental extract contains stem cells and the placental mesenchymal stem cells that serve to attenuate the T- cell activity in immune reactions .The placental mesenchymal stem cells prevent proliferation and release of cytokines by the T helper- 1 cells while inducing the expression and secretion of T helper- 2 cells. Also, these placental mesenchymal stem cells induce the differentiation of the T regulatory lymphocytes and Th2 polarization. All that is performed by direct contact of the placental mesenchymal stem cells with the T cells. Thus the placental mesenchymal stem cells serve to attenuate T cell activity. Moreover, the placental extract possesses various physiologically powerful growth factors that promote the growth of various body cells such as; liver parenchymalcells; nerve cells; lungs, skin, cornea and tracheal epithelial cells; smooth muscle cells; cartilage cells; vascular endothelial cells and, most importantly, stem cells[14-23]. Postnatal stem cells are found in almost all body tissues including dental tissues[11, 20, 21]. To date, four types of human dental stem cells have been characterized and isolated :

- Dental pulp stem cells (DPSCs)
- Stem cells of the apical papillae (SCAP)
- Periodontal ligament stem cells (PDLSCs)
- Stem cells from human exfoliated deciduous teeth(SHED)[26-30]

Growth factors are extracellularly secreted signals that govern morphogenesis and organogenesis during epithelial mesenchymal interactions. They regulate the division and differentiation of stem cells to the desirable cell type. Also, they mediate key cellular events in tissue regeneration as cell proliferation chemotaxis. Moreover, some growth factors increase the stem cells number suchas; the platelet – derived growth factor (PDGF); fibroblast growth factor (FGF); insulin- like growth factor (IGF); epidermal growth factor and colony- stimulating factor (CSF)which elevate the immune strength. Other growth factors modulate the humoral and cellular immune responses, and others are important regulators of angiogenesis, as the vascular endothelial growth factor (VEGF). Another group of growth factors are important for tissue regeneration/ engineering as transforming growth factors alpha and beta[17-24;31-34].

A very distinct family of growth factors implicated in tooth development and regeneration; are bone morphogenic proteins (BMPs) which have been described as an important biologic tool for dentin regeneration. Recombinant human BMP – 2 has been reported to stimulate differentiation of adult pulp stem cells into odontoblasts and enhance hard tissue formation in vivo[17-24; 31-34]. In accordance to the immunopathological status described before, the odontoblasts, being situated at the periphery of the dental pulp, form the first line of defense. Following pathogenic recognition, the odontoblasts and immune cells (found in the pulp), induce a multitude of signaling molecules to control the immune reaction. It starts with the odontoblasts release of various cytokines (IL-1a, IL-1b, IL 4, IL 6, IL 8, IL 10); that are known to control different aspects of the inflammatory response according to their levels and profiles[22].The immunomodulatory role of the placental extract is as follows; the placental mesenchymal stem cells (PMSCs) prevent proliferation and release of cytokines by T- helper – 1 cells and, at the same time, induce the expression and secretion of T- helper- 2 cells. Meanwhile the (PMSCs) induce the differentiation of T regulatory lymphocytes, Th2 polarization (accompanied by increased levels of IL 4 and IL 10). Thus the (PMSCs) of the placental extract serve to attenuate T cell activity[22,23]. This immunomodulatory action of the placental extract together with the regenerative role of the growth factors found in the placental extract (as described before); induce a therapeutic action that results in tissue healing and powerful regeneration and proliferation and, thus, dentinogenesis. Currently, the human placental extract have been approved for clinical use[17-20]. Also, it has been reported as safe with no significant side effects or contraindications[17-20]. In this study, the placental extract was used along with a local drug delivery system in order to transfer the loaded placental extract to the target site (dental pulp) as well as to provide a sustained and controlled release of the placental extract. That, consequently, would result in increased efficacy and local concentration of the placental extract. The local drug delivery systems have been reported to act as targeted drug delivery vehicles[35-38].The drug delivery system used in this study included three nano fibered polymers; chitosan, polylactic-co–glycolic acid and hydroxyl propyl methyl cellulose.

The electrospinning process resulted in a more controllable viscosity of the polymers and, more important, produced the nanofiber state of the polymers which could incorporate the placental extract and allow its release in targeted, sustained and controlled manner. The gel consistency of the polymers contributed, in part, to the sustained and controlled release of the placental extract .This is due to the fact that gels,due to their high swelling would limit the possibility of a burst release of the drug [the placental extract]. Chitosan was used because it is a naturally occurring polymer showing high bioactivity, biocompatibility and biodegradability. Also, chitosan, due to its high stretch characteristics and production of thinner nanofibers, could

incorporate a high content of drug [placental extract] molecules[39,40]. The polylactic-co–glycolic acid is a synthetic polymer and was used to provide the blend of polymers better mechanical properties. The hydroxy propyl methyl cellulose polymer was used to impart a desirable viscosity to the blend of polymers used. The use of a blend of three types of polymers was intended in order to potentiate the therapeutic effect of the local drug delivery system used. Regarding the sub-group(Pa), the resultant electrospun bundle of nanofibers(nanoyarn), was manually and thoroughly twisted and mixed with the placental extract.

Meanwhile, for the subgroup (Pb), the electrospun bundle of nanofibers was cut into pieces that were, eventually, soaked in the placental extract for 10 min. The comparison between the two subgroups was to find out which method of incorporation of the placental extract was more effective. This study is evidence – based. It was conducted in vivo and the decision to use human placental extract to induce repair and regeneration of the pulp tissues and cells for the individual patients was set according to their oral environment rather than treating all patients similarly. Also, our therapeutic treatment included strategies that placed the patients into a healthy balance; examples include; the healthy meticulous safety measures followed during manufacturing of the placental extract product (according to the manufacturer); and the selection of patients who demonstrated good oral hygiene and who had no medical contra indications for dental treatment. The selected patients for this study were males as well as females to rule out any factors related to gender. The patients included in this study were family members, friends and business associates in order to facilitate recall and follow up. A preliminary medical examination, for all patients included in this study, was required to rule out any medical contraindications for dental treatment. Also, a preliminary dental examination was conducted to assess the oral hygiene status and to ensure pulp vitality of the selected teeth for treatment. Regarding group (P), that received the placental extract therapy, after two weeks and at all evaluation time periods (three and six months and two years), thirty seven cases of forty were successful. Those cases, clinically, demonstrated proper function as well as complete absence of clinical signs and symptoms as swelling, pain, tenderness, tooth mobility and fistula. Meanwhile, radiographically, those cases showed appropriate function with normal periapical tissues and normal periodontium. Also, there was complete absence of any loss of lamina dura, widening of the periodontal ligament, signs of pathologic internal or external root resorption and periapical and interradicular radiolucency. In addition to that, all those cases demonstrated clear dentin bridges. All that was indicative of appropriate cell regeneration and dentinogenesis. This success rate is attributed to the immunomodulatory action of the human placentalextract used in addition to its powerful regenerative action due to its possession of numerous growth factors (as described before). This induced a therapeutic action that resulted in tissue healing and powerful regeneration and proliferation and, thus, dentinogenesis. In addition to that, the placental extract could govern morphogenesis and organogenesis, improve basal cell metabolism, activate the immune system and improve the blood circulation [17-23]. Regarding the success of the therapeutic treatment of cases belonging to group (P), the recorded results of the subgroups (Pa) and (Pb); demonstrated no statistically significant differences which indicated that both methods of administration of the placental extract were equally successful. The success rate of cases belonging to the control group (C); was, significantly, less than that of group (P) which provides strong evidence of the therapeutic powerful immunomodulatory and regenerative role of the human placental extract used. The results of our study were found successful over a two years time period. Further research over comparatively longer time periods may be tried.

CONCLUSION

The human placental extract is a therapeutic agent that demonstrates an amazing regenerative power. The local drug delivery systems are very successful in producing targeted and controlled release of therapeutic agents.

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