RESEARCH ARTICLE

BONE SUBSTITUTE VERSUS STEM CELLS IN REGENERATION OF ALVEOLAR BONE DEFECTS IN HUMANS: SYSTEMATIC REVIEW.

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Introduction:

The repair and regeneration of alveolar bone defect is a major problem encountered oral and maxillofacial field. Bone loss is mainly produced by different causes and diseases including congenital and degenerative diseases, traumas as well as surgical procedures. This diseases might led to functional, social, and esthetics problems especially in old. Critical conditions of the alveolar bone due to periodontitis, extraction, or trauma provoke decrease in the alveolar ridge volume due to bone atrophy. Bone atrophy might produce changing in interarch relationship in vertical, transverse, and sagittal planes. The defective alveolar bone could be augmented by different techniques including: onlay and inlay grafting, ridge expansion, distraction osteogenesis, and guided bone regeneration (GBR).

Keywords:- bone regeneration, alveolar bone defect, bone substitute, stem cells.
Stem cells are primitive cells found in all multicellular organisms characterized by self-renewal and have the capability of differentiation into any mature cell type. Stem cells have the potential for regeneration and repair of damaged cells. According to the origin and differentiation potential of the stem cells, there are two main types, embryonic stem cells derived from fetal tissue and adult stem cells that can be harvested from bone marrow and other sources such as liver, umbilical cord, placenta, adipose tissue, synovial membrane, amniotic fluid and teeth. Stem cells have multipotency to differentiate and develop into various types of tissues as adipose, cartilage, and bone (7-11).

Dental pulp (DPSCs) is a niche housing neural-crest-derived stem cells. It is easily available with limited morbidity after collection. DPSCs are capable of differentiating into osteoblasts that secrete abundant extracellular matrix that can build a woven bone in vitro. It is also capable of forming a complete and well-vascularized lamellar bone after grafting. Dental pulp could be considered an interesting and possibly an important source of autologous stem/progenitor cells that are ready for use for therapeutic purposes, as the repair/regeneration of craniofacial bones (12-17).

The ideal graft material should not only be a bone substitute but a bone regeneration material that is completely resorbed simultaneously with the formation of new bone. Its decomposition products should be reused for building new bone (18). It should serve as space keeper preventing invasion of soft and connective tissue and should not carry any immunological risk. Autogenous bone grafts which are still regarded as a gold standard appear to be ideal but their availability and storability is limited and secondary surgical sites with all related risks are still founded (>5). Allogenic or xenogenic grafting materials do not require secondary surgery. They are readily available and can be stored but the risk of immunological reaction due to foreign protein and transmission of viral or other infections cannot completely be prevented, making their use doubtful. The resorption of xenogenic materials has been the subject of controversy and may be identified histologically after many years (22-25). Synthetic calcium phosphate as hydroxyapatite, alpha and beta tri-calcium phosphate are artificial, sterizable, free from any risk of material induced infections and easily available. The gradual dissolution and resorption of the synthetic bone substitute in physiologic environment occurs predominantly through physiochemical means without osteoclast activity. This procedure leads to interlocking porosity, allowing an invasion of fluids, migration of cells and ingrowth of vessels and newly formed bone, thus being osteoconductive (26, 27).

Human dental stem cells that have been isolated and characterized derived from different sources include dental pulp stem cells (DPSCs), human exfoliated deciduous teeth, stem cells from apical papilla (SCAP) and periodontal ligament stem cells (PDLSCs) (28). In the last ten years stem cells have gained more interest because of their high differentiation potential and their availability. Different types of stem cells represent a potential key component in autologous graft for bone regeneration. In contrast, bone substitute materials that were commonly used for reconstruction of alveolar bone defect including autogenous bone graft, allograft, xenograft and synthetic bone materials. The aim of the present investigation was to systematically review and assess all relevant literature concerning the regeneration of alveolar bone defect using bone substitute versus stem cell in humans.

Materials and Methods:-
Search Strategy:
Identification of studies to be considered for inclusion was based on a search strategy for each electronic database PubMed, Booksc.org, LILACS, and Google scholar. In accordance with guidelines of the preferred reporting items for systematic reviews and meta-analysis (prisma) statement and Cochrane handbook from January 1990 up to April 2017. LILACS search lead to articles in different language so it was excluded. The search used the following keywords: (bone regeneration OR alveolar bone defect OR bone substitute OR dental pulp stem cell OR adipose derived stem cell OR autograft OR allograft OR alloplast OR xenograft) that was combined with manual search. The search was limited to randomized clinical trials involving human subjects with restrictions to English.

All original research and review articles bibliographies were identified to be relevant to the scanned subject for any possible additional studies. Title and abstract of identified studies were screened by two reviewers for eligibility (AK and WA). Consensus was obtained by discussion or consultation with the third reviewer (MD). The detailed search sequence presented in table (1).
Selection criteria:
All randomized controlled clinical trial (RCT) assessing stem cells and bone substitute in regeneration of alveolar bone defects were included. No limitation was positioned in regard the number of patients treated. Studies published between January 1990 and April 2017 were included.

Inclusion criteria:
1. Human studies.
2. Bone substitute studies.
3. Stem cells studies.
4. Treatment outcomes that was clearly reported by the authors.

Exclusion criteria:
1. Articles in language rather than English.
2. Animals study
3. Case report.
4. Review paper.
5. Paper including periodontal or intra-bony defect.
6. Paper with unclear patient grouping.
7. Technical reporting.

Table 1: Detailed search sequence of the 4 articles used in this review

| Paper name | Author (year) | Study design | Subjects | Duration | Gender | Age in years (mean / range) | Funding | Groups | Test group | Control group | Original authors’ conclusions |
|------------|---------------|--------------|----------|----------|--------|-----------------------------|---------|--------|------------|-----------------|-----------------------------|
| Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial. | Kaigler, et al 2013 USA | Tissue repair cells isolated from bone marrow were investigated to reconstruct localized craniofacial bone defects Oral implants were installed, subsequently restored, and functionally loaded with tooth restorations. | 24 | Both ages 20–70 | | | No | TRC was placed onto gelatin sponge 12 a bone marrow aspiration of the posterior ilium under conscious sedation and local anesthetic | sponge, soaked in 1 ml sterile saline GBR 12 | -Clinical, radiographic, tomographic, and histological measures demonstrated that TRC therapy accelerated alveolar bone regeneration compared to GBR therapy. -TRC treatment significantly reduced the need for secondary bone grafting at the time of oral implant placement with decrease in implant bony dehiscence exposure (residual bone defects) as |
Transplantation of TRCs for treatment of alveolar bone defects appears safe and accelerates bone regeneration, enabling jawbone reconstruction with oral implants. The results from this trial support expanded studies of TRC therapy in the treatment of craniofacial deformities.

Human Mandible Bone Defect Repair the Grafting of Dental Pulp Stem/Progenitor Cells and Collagen Sponge Biocomplexes

Aquino et al 2009 Italy

Biocomplex (DPCs) and a collagen sponge scaffold used for (OMF) bone tissue repair in patients requiring extraction of their third molars.

3 months

17 Both No

(DPCs)and collagen sponge scaffold was used to fill extraction site.

Collagen sponge used to fill the extraction site.

(i) DPCs can be used for OMF bone repair;
(ii) The use of DPCs on appropriate scaffold produces an efficient biocomplex;
(iii) Collagen sponges can be considered an optimal support for DPCs.

Role of platelet-rich plasma in combination with alloplastic bone substitute in regeneration of osseous defects

Singhet al 2011 India

Evaluate the alloplastic bone substitute for its osteogenic potential with or without PRP

180 days

23 Both No

Group B had 13 (56.5%) patients whose osseous defects were filled with ABS with PRP

Group A had 10 (43.5%) patients whose osseous defects were filled with ABS mixed in normal PRP accelerates vascularization of the graft, improves soft tissue healing, reduces postoperative morbidity and enhances bone regeneration. Advantages of
Three Years after Transplants in Human Mandibles, Histological and In-Line Holotomography Revealed That Stem Cells Regenerated a Compact Rather Than a Spongy Bone: Biological and Clinical Implications

| Giuliani et al 2013 Italy | DPCs capable of producing bone when seeded on collagen scaffolds and can be used for repair of human mandible defects. 3 years | 7 | Yes | DPCs seeded in collagen sponge scaffold treated mandible at extraction site | Collagen sponge inserted at extraction site |
|---------------------------|-------------------------------------------------------------------------------------------------|---|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|

(a) DPCs seeded on a collagen scaffold repair bone.
(b) Three years after grafting in mandibles, revealed that regenerated bone is uniformly vascularized and qualitatively a compact type, rather than a cancellous type that is physiological for the area.
(c) Regeneration of compact bone occurs because DPCs do not follow the local signals of the surrounding environment.

Saline (NS) using an autologous PRP include no risk of cross-reactivity, immune reaction or disease transmission. In addition, the use of PRP improves handling characteristics of particulate graft material and affords easier packing into a grafting site thus, facilitating space maintenance and potential for bone regeneration.
spongy bone.
(d) Clinical advantages afforded by the grafting of autologous DPSCs more significant than the disadvantages arising from regeneration of a bone type that is not normally present in the area treated.

Risk of bias within studies: For RCT studies, according to Cochrane Risk of Bias Tool, all studies were judged as low risk of bias for, selective reporting and intention to treat. Regarding random sequence generation and Allocation concealment, 2 studies: Kaigler et al 2013 and, Singh et al 2011) (table 1) were judged as low risk of bias while it was judged as unclear for, Aquino et al 2009 and Giuliani et al 2013 study (table 1) as it did not mention type of randomization used. Regarding Blinding of outcome assessment, 3 studies (Aquino et al 2009, Singh et al 2011 and: Giuliani et al 2013) were judged as unclear as there was no any mention about blinding of the assessor or statistician while it was low risk of bias for Kaigler et al 2013 study. Singh et al 2011 consider as high risk of bais for incomplete result and other 3 studies were considered overall low risk of bias. Figure (1) represents risk of bias summary: review authors’ judgments about each risk of bias item for each included RCT study while Figure (2) represents risk of bias graph: review authors’ judgments about each risk of bias item presented as percentages across all included RCT study.

Figure 1: Risk of bias summary: review authors’ judgments about each risk of bias item for each included RCT study
Figure 2: Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included RCT studies

Paper 1: Kaigler et al 2013
paper2: Aquino et al 2009
paper3: Singh et al 2011
paper4: Giuliani et al 2013

Results:
Out of the initial search that yielded 153 studies, 20 were considered potentially relevant for the present study, out of which 4 were, finally, selected. Figure (3) represents the flow chart for the study. The excluded studies before final inclusion was summarized in Table (2) for reasons.

The final included studies were four (Kaigler et al 2013, Aquino et al 2009, Singh et al 2011 and Giuliani et al 2013). The heterogeneity between trials prevented meta-analysis. Rather, a descriptive analysis of the reported studies was performed. Table (3, 4, 5, 6) represents summary of findings.
Figure 3: Prisma flow chart for the study.

Table 2: Summary of the excluded studies and the reason for their exclusion:

| References          | Reasons for exclusion          |
|---------------------|--------------------------------|
| 1 Grunder et al 2011| Periodontal defect            |
| 2 Snyder et al 2012 | Case report                    |
| 3 Friedmann et al 2002 | Problem in patient grouping    |
| No. | Author et al Year | Description |
|-----|-------------------|-------------|
| 4   | Walters et al 2003| Periodontal defect |
| 5   | Rodrigues et al 2011| Periodontal defect |
| 6   | kasaj et al 2008 | Periodontal defect |
| 7   | Lekovic et al 2002| Periodontal defect |
| 8   | Yukna et al 2000  | Periodontal defect |
| 9   | Cetinkaya et al 2006| Periodontal defect |
| 10  | Camargo et al 2005| Periodontal defect |
| 11  | Lekovic et al 2012| Periodontal defect |
| 12  | Camargo et al 2002| Periodontal defect |
| 13  | Gupta et al 2011  | Periodontal defect |
| 14  | Matos et al 2007  | Periodontal defect |
| 15  | Yamamiya et al 2008| Periodontal defect |
| 16  | Grimm et al 2014  | Case report |

**Table 3:** Summary of findings for Paper 1: Kaigler et al 2013: Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial.

| Parameters                                | Test group                          | Control group                          |
|-------------------------------------------|-------------------------------------|----------------------------------------|
| Bone Density and Residual Bone Defects     | 80.1 ± 2.0% (p = 0.01)              | 74.6 ± 3.3%                            |
| photographic images                        | Bone-like appearance clinically, was denser, and demonstrated high vascularity during biopsy harvest. | Highly vascular and fibrous, and most specimens were notably soft during biopsy harvest |
| Biopsy Analyses: micro-CT analysis (μCT) and Histomorphometry | Bone volume fraction (BVF) 28 ± 8% (p = 0.08). Bone mineral density (BMD) (195.0 ± 63.3 mg/cc) p = 0.1 | 13 ± 6%, (85 ± 46.3 mg/ cc). |
| % bone area/tissue area (BA/TA)            | At 6 weeks 28.8 ± 9.1% (p = 0.10)   | At 12-week 35.2 ± 8.9%, 19.6 ± 4.2%    |
|                                           | At 12-week 35.2 ± 8.9%              | 35.1 ± 3.2% and                        |

**Table 4:** Summary of findings for Paper 2: Aquino et al 2009. Human mandible bone defect repair the grafting of dental pulp stem/progenitor cells and collagen sponge bio-complexes

| Parameters                                | Test group                          | Control group                          |
|-------------------------------------------|-------------------------------------|----------------------------------------|
| Clinical                                  | 7 Day: both group same- slight edema- no postoperative pain-normal healing with no scar | Not seen |
| Bone level Probing depth                  | 30 Day: Cortical bone reach to level of CEJ of 2nd molar | Not seen |
3 Month: cortical bone level higher 6.2±2.3 mm 4.4± 1.2 mm

Radiological 30 Day: High rate of mineralization

Histology Well organized and well vascularized bone with a lamellar architecture surrounding the Haversian channels Immature, with fibrous bone entrapped among new lamellae, incomplete and large Haversian channels and evidence of bone reabsorption

Immune florescence analyses Significant differences were observed for BMP-2 and VEGF expression: they were expressed at much higher levels (p<0.001) in the T group with respect to the C group

One year Bone regeneration Higher in T group with p<0.01, vs C group for all patients except N. 7.

**Table 5:** Summary of findings for paper3: Singh et al 2011: Role of platelet-rich plasma in combination with alloplastic bone substitute in regeneration of osseous defects

| Parameters                      | Test group                                                                 | Control group                                      |
|---------------------------------|----------------------------------------------------------------------------|---------------------------------------------------|
| Clinical                        | Healing was significantly higher T group (ABS + PRP) as compared with C (ABS + NS) Pain: no significant difference between two groups at any time interval (P >0.05) Postoperative swelling: no significant difference between two groups at any time interval (P >0.05) Infection and graft rejection: no significant difference between two groups at any time interval (P >0.05) |                                                     |
| The scintigraphic               | 3 patients with bilateral defects showed increased tracer uptake in region of osseous defect filled with ABS with PRP. The tracer uptake was 1.36 times higher in 1 patient, 1.30 times in second and 1.79 times in third patient in region of osseous defect filled with ABS with PRP |                                                     |

**Table 6:** Summary of findings for paper4: Giuliani et al 2013: Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications

| Parameters                      | Test group                                                                 | Control group                                      |
|---------------------------------|----------------------------------------------------------------------------|---------------------------------------------------|
| Clinical                        | Both group same normal                                                    | Less hard Drilling force 21N-cm                    |
| Bone regeneration               | Harder than C Drilling force 36N-cm                                       | Less hard Drilling force 21N-cm                    |
| Bone level                      | 6.3 + 2.1 mm                                                               | 4.5+ 1.4 mm                                       |
| Probing depth                   |                                                                           |                                                   |
| Radiological                    | High rate of mineralization                                               | Cancellous (spongy) bone type interrupted lamellae surrounding numerous large marrow-filled spaces arranged in a more or less regular pattern |
| Histology                       | compact bone architecture                                                 | Cancellous (spongy) bone type interrupted lamellae surrounding numerous large marrow-filled spaces arranged in a more or less regular pattern |
| Histomorphometric analysis      | BV: 1.10 + 0.3 (x10⁸) μm³ ≤ .001                                          | 0.53 + 0.31 (x10⁸) μm³                            |
|                                 | BS/TV (%): 79.8 + 10.3 ≤ .01                                              | 47.6 +7.6                                         |
| Synchrotron Radiation-based Holotomography | more compact bone                                                  |                                                   |

**Discussion:**
Bone substitute and stem cells can be used as alternatives for regeneration and restoration of damaged and lost alveolar bone defects in humans in oral and maxillofacial field. Clinical analyses of the bone defect after replacement and augmentation demonstrated that there was bone regenerative response determined radiographically, and
histologically. Stem cells is easily accessible with limited morbidity, capable of differentiating into osteoblasts and producing well-vascularized lamellar bone. Thus, it could be used for bone regeneration for craniofacial bones. Bone substitute can restore and maintain facial bone and help soft tissue support with reasonable aesthetics, easy to use and handle, with favorable cost and time advantages and adaptability to a various oral and maxillofacial areas. Review of literature revealed that both bone substitute and stem cell have several advantages and disadvantages and, hence, the research hypothesis was established to compare bone substitute versus stem cell in regeneration of alveolar bone defects in humans.

Alveolar bone defects is an aesthetic and functional problem for many patients. Bone defects can be resulted from oncologic surgery, traumatology, and implant surgery. Reconstruction of such a defect represent clinical challenges. Different modalities have been proven its effectiveness in restoring the bony defects. These modalities included bone substitute and stem cells with its different sources, advantages, and disadvantages. However, up to date, no evidence-based approval for either to replace the bony defects with conventional modalities including bone substitute or replace it with the recent technique including stem cells and its variety. The trials presented in this review agreed that both modalities are efficiently used for reconstruction of alveolar bone defects but further discussion is recommended to advocate one over the other.

Frequency of bone grafting is the second most frequent tissue for transplantation, worldwide. Bone substitutes with different types and forms considered the best method for replacement the different types and sizes of alveolar bone defects being biocompatible, easily molded into the bone defect. Also bone substitute is considered osteoconductive, osteoinductive, thermally nonconductive, sterilizable, as well as readily available at a reasonable cost. This concept in agreement with Mironet al and Pryor et al.

Several studies proved that autografts are the gold standard method in bone substitution for several reconstruction procedures. The autografts possess an osteoconductive and osteoinductive properties, contain many growth factors and osteogenic cells for bone formation as well as slowly replaced by newly formed bone. The disadvantages of autografts was the second surgical donor site with possibility of post-operative pain and complications; infection, fracture, or neurovascular injury, as well as cosmetic deformity, and longer operative time. Tomford and Lomas et al recommended the use of allograft as a suitable alternative to autogenous bone graft. But, the disadvantages are costs, difficult procedure (tissue processing, harvesting), and its mechanical resistance limited the process of osteoinduction as well as it has risk of infection transmission.

Xenografts can be used for reconstruction of alveolar bone defects being osteoconductive with good mechanical properties, low costs and easy available. Alloplastic material in form of hydroxyapatite most commonly due to its osteoconduction, hardness, and acceptability by bone. However, calcium carbonate was completely resorbed in short time that lead to bone fracture. Tricalcium phosphate in combination with hydroxyapatite giving the effect of both, resorbable and stoelconduction.

Despite that bone substitute has been the brilliant technique for reconstruction of defective alveolar bone, stem cells start to gain importance in that field because it possesses superior osteogenic ability. Using of stem cells for reconstruction provides benefits not only to oral and maxillofacial surgeon but also for the patients. Patient-centered outcomes are the main target for researches in the last decade.

Concerning prevention of facial deformity as one of the complication resulted from defective alveolar bone the osteoinductive stem cells based therapies can be used to improve and accelerate the clinical outcomes. One of the advantages of using stem cell is more predictable regenerative outcomes and improved esthetics. Local immune responses by the host cells against the stem cell are highly relevant in regenerative medicine. Mesenchymal stem cells may be applicable to suppress the local immune response during transplantation to attain ideal tissue regeneration. Stem cells and tissue engineering therapies are expected to provide a novel capability to regenerate large defects in periodontal tissues and alveolar bone, and to ultimately replace the lost tooth itself.

The present review ascertained that stem cells hold several advantage over bone substitute in reconstruction procedures. This idea was supported by the opinion of Watt and Graziano. Being autologous and harvested form a natural source, easy and faster method to repair and regenerate damaged tissues with low-risk and effective therapeutic strategy, exhibits minor morbidity of the collection site, free from diseases experienced by disease transmission, and no need for secondary bone grafting procedures in small defect. However, on grafting a defective
area with stem cells, it must be taken into consideration their behavior as it might be relatively variable on the differentiation process. It may be affected by their origin rather than by the local signals arise from the treated area. The clinical advantages afforded by the grafting of autologous stem cells may be more significant than the disadvantages arising from the tissue that regenerated.

A doubt remains regarding the use of stem cells to find adverse effects. Stem cells are usually introduced to find common and intended outcomes, whereas adverse effects tend to be less frequent and unintended. Trials upon which this review is based might be useful to detect systematic adverse effects as the type of regenerated tissues but might be less advantageous as mentioned above. In contrast, in the 4 human clinical trials that met the eligibility criteria for this systematic review and encompassed 71 adult subjects in various clinical settings between January 1990 and April 2017, all the publications concluded that the use in stem cells or bone substitute for reconstruction of alveolar bone defect produced uncounted safety outcomes. (17, 57, 59)

The finding could be categorized in one or more ways: stem cells and bone substitute can be used for alveolar ridge reconstruction; dental pulp stem cells seeded on an appropriate scaffold as collagen can repair bone and produces an efficient biocomplex, tissue repair cells isolated from bone marrow accelerated alveolar bone regeneration and reduced the need for secondary bone grafting at the time of dental implant placement and finally the use of platelet rich plasma with autogenous or alloplastic bone substitute can accelerate vascularization of the graft. improves soft tissue healing, reduces postoperative morbidity and enhances bone regeneration. Besides, it improves handling of graft material particles and help to manage and packing it easily into the proposed graft site therefore, assisting maintenance of the space with rapidly bone regeneration.

Conclusion:-
Clinical studies that encountered in this research are too limited in number and so it displays small sample sizes. It is clinically heterogeneous with nosolid conclusion can be reached. Investigators should pay their attention to this remarkable subject and investigate it deeply. Each kind of stem cells should pull attention of researchers in oral and maxillofacial field to close obvious, yet important, research gaps of lack of enough randomized clinical trials that can be more trusted and get a standard evidence based clinical practice. Within the limitations of this review, it can be concluded that stem cells can be used as a safer and effective treatment modality to provide reconstruction of small maxillofacial bone defects. However, bone substitute is higher cost-effective procedure reconstruction of such defects. The easily availability and less disease transmission with less morbidity of the donor site and cost of surgical approach to harvest stem cells make this technique superior to higher cost alloplastic bone graft. Further studies with a larger study samples and a longer follow-up period would be desirable with special concern on technique is recommended for larger bone defect site.

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