Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-149 Vol 6 Issue 2, 2014

Research Article

THE ROLE OF PROTEIN DEFICIENCY IN THE HEALING OF MANDIBULAR FRACTURES IN RABBIT MODEL

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Received: 23 Jan 2014, Revised and Accepted: 03 Mar 2014

ABSTRACT

Treatment of mandibular fractures still represents a tremendous challenging issue for the maxillofacial surgery due to the biological and biomechanical conditions that are unfavorable for fracture fixation and bone healing. Calcium and protein are essential building blocks of bones, which are needed in bone healing process.

Objective: To investigate the effect of protein diet on the healing phase and inflammatory degree in the healing process of fractured mandible.

Methods: Twenty-four skeletally immature male New Zealand white rabbits aged between 3 and 5 months, with a mean weight 2.63 kg ±.35 kg were used. The rabbits were equally divided into three groups. Group A rabbits were given the rich protein diet; group B rabbits were the control group and were given a normal diet while group C rabbits were submitted to a restricted protein diet to form the undernourished group. The rabbits in all groups were subjected to approximately the same transverse fracture created under general anaesthesia in the mandible at the anterior midline. A rabbit from each group was sacrificed weekly for 8 weeks post-operatively. Harvested mandibles of the region of interest were subjected for histological tissue processing (decalcification and sectioning process). The mandible was carefully dissected from the cranium with conservative dissection of the soft tissues and muscles attached on the mandible around the area of the fracture. The histological study comprised of the fracture site evaluations were weekly observed and recorded.

Results: One-way analysis of variance was used to investigate the differences between the mean of healing stages among the three groups of rabbits. The results showed a significant difference between the healing phase of the 'Rich Protein' group compared to the healing phase of the 'Restricted Protein' group.

Conclusion: Rich protein diet could accelarte with high quality of the healing process mandibular bone fracture.

Keywords: Mandible, Protein energetic undernutrition, Repair, Mandibular fracture bone repair.

INTRODUCTION

The mandible, the largest and strongest bone of the face, serves for the reception of the lower teeth. Mandibular fractures have a high incidence account 2–3 times more frequently than other facial bones for 36% to 59% of all maxillofacial fractures, thus representing the first or second most common facial injuries [1]. Only the nasal bones are fractured more often as a result of facial trauma [2]. Fractures of the mandible constitute the bulk of the trauma treated by an oral and maxillofacial service. Because of less bony support the mandible is considered vulnerable particularly due to prominent position on the face [3].

Generally fracture healing involves complex processes of cell and tissue proliferation, migration and differentiation [4]. Growth factors, inflammatory cytokines, antioxidants, bone breakdown (osteoclast) and bone-building (osteoblast) cells, hormones, amino acids, and uncounted nutrients are recognized as the main players of complex processes process fracture healing [4]. It is complex tissue that requires many nutrients. Immediately upon fracture, a blood clot forms, allowing the influx of inflammatory, clean-up cells to the wound area [5]. Fracture healing can be divided into three phases. The inflammation phase is the first stage of healing [6]. This is followed by a cytokine cascade that brings the repair cells into the fracture gap. These cells immediately begin to differentiate into specialized cells that build new bone tissue (osteoblasts) and new cartilage (chondroblasts). Over the next few months, these cells begin the repair process, laying down new bone matrix and cartilage [5]. At this initial stage, osteoclast cells dissolve and recycle bone debris [5]. The second, reparative stage begins about two weeks after the fracture occurs. In this stage, proteins produced by the osteoblasts and chondroblasts begin to consolidate into what is known as a soft callus [6]. This soft, new bone substance eventually hardens into a hard callus as the bone weaves together over a 6- to 12-week time period [6]. The final step of fracture repair is known as the remodeling phase. At this stage the callus begins to mature and remodel itself [6]. Woven bone is remodeled into stronger lamellar bone by the orchestrated action of both osteoblast bone formation cells and osteoclast bone resorption cells [6]. Each stage of the fracture healing process brings with it increased nutritional demands [7]. For starters, the whole process requires a great deal of energy-which is generally supplied through the intake of calories in food [7]. Next, healing requires the synthesis of new proteins, which is dependent upon an ample supply of amino acids derived from dietary proteins [7].

An adequate blood supply is also mandatory for fracture healing, so anything that diminishes blood flow (such as smoking or poor circulation) slows the healing process. Also, the trauma of the fracture itself creates a biochemical burst of pro-oxidants (free radicals), causing oxidative stress that can overwhelm the body's antioxidant reserves. Angiogenesis consider a crucial step in healing of fracture by formatiom new blood vessels resulting in improvment blood supply at defect site [8]. Vascular endothelial growth factor have a driving force behind endothelial cells. Act directly in VEGFreceptor-2- found in endothelial cells which responsible for formation new blood vessels considered essential to success healing of fracture [8]. If fracture bone that are compromised by poor blood supply heal slower than well perfused wounds [9]. Protein-energy malnutrition affects approximately half of the population worldwide, especially in developing countries and it affects virtually every organ system [10]. Fracture healing consists of a complex series of cell events demanding a high protein synthesis rate [5]. The damaging effects of protein undernutrition on callus formation, leading to nonunion and intermediate bone healing, have been experimentally demonstrated [11,12]. Many systemic and local factors influence fracture healing; nutritional state including vitamins, minerals, trace elements supplementation and pharmacological agents [13]. The aim of this study was to evaluate the effect of the daily intravenous administration of 10% of protein with addition of calcium, fluoride and vitamin C as dietary supplementation on treated mandibular bone fracture healing in rabbits.

MATERIALS AND METHODS

This study was approved by the research ethics committee at the College of Dentistry, University of Baghdad and follows the council for international organization of medical sciences ethical code for animal experimentation [14]. Lipovenoes® 10% PLR were purchase from Fresenius Kabi Deutschland GmbH, Bad Homburg v.d.H., Germany. Pharmaceutical form: Emulsion 10% for intravenous infusion for parenteral nutrition, similar phospholipid/triglyceride ratio as found in 20% emulsions. For adaptation in animals' house, rabbits were kept under veterinary and operator supervision for a minimum of 1 week before surgery to allow for acclimatization to the laboratory environment. Twenty- four young male aged between 3 and 5 months New Zealand white rabbits, College of Veterinary, Baghdad University were used. The animals were kept in separate metallic cages. In each cage one animal feed with standard ration and water. Lipovenus 10% of proteins and vitamins and minerals maintained according to the American Institute of Nutrition to AIN-93G recommendations for rodent diets were given to group A [15]. The diets of rich protein group were administered in powder form for 8 weeks postoperatively in addition I.V injection of Lipovenus 10% protein dose 0.5cc twice daily [16]. Control group B diets with green food and grains 25g of high fibre/low protein dry food per 1kg of body weight with water. While restricted protein group C given green food only (green leafy vegetables) with water.

Experimental technique

All surgical equipments were sterilized in an autoclave. Sterile gowns, gloves, surgical masks and theatre caps were used. The site of operation for mandible was prepared for surgical work. Rabbits were positioned ventro-dorsally, anaesthetized preoperatively with intramuscular administration of ketamine (50 mg/ml), 35 mg/kg and xylazine (100 mg/ml), 5 mg/ kg. To maintain the extent of anaesthesia, further doses of ketamine hydrochloride were given at 30-minute intervals during the operation. Administration of local anaesthetic solution 1 ml of 2% lidocaine /epinephrine 1:80 000 (Astra, USA) was injected at the surgical site (Incisional line block). Cardiopulmonary functions were monitored by pulse oximeter during the operation by an experienced veterinary physician. Skin incision was done from the midline of the mouth and extended inferiorly with the rabbit's head hyper-extended. Dissection using a haemostat was carried out through subcutaneous tissue. Periosteal flap including muscle was raised and the lateral aspect of mandible was exposed. The mental foramen with the emerging inferior alveolar nerve located immediately anterior to the first premolar tooth were identified and dissected (Fig. 1). A transverse osteotomy induced at the anterior midline of the mandible using micromotor hand piece with speed of 20000 rpm with plenty irrigation cooling. Small superior and inferior cuts were done in the proposed osteotomy, a straight body incomplete osteotomy cut was made. Great care was taken to preserve the inferior alveolar neurovascular bundle. The osteotomy was then completed with a chisel and mallet. Two holes were made across the two segments of the fractured bone to pass the stainless steel wire (gauge 0.5 mm) through and fixed the fracture. The wire then was tightened, reflected laterally (inward) (Fig. 2). Haemostasis was ensured. Periosteum and muscle were reapproximated. The wound was then closed in layers using 4-0 Vicryl, cleaned with povidone iodine antiseptic and neomycin ointment (antibiotic) was applied. Postoperatively each animal was observed closely by a veterinary technician and operator until regained consciousness at which time it was returned to its individual cage. Collars were worn by all animals to prevent suture chewing. The animals were monitored weekly for 8 weeks postoperatively. All animals were given an intramuscular injection of Baytril (Enrofloxacin), 10 mg/kg/day (Bayer, Shawnee Mission, KS, USA) once daily for 7 days. Tramadol hydrochloric 2 mg/kg once daily was given for pain relief for 7 days. The rabbits' weight was recorded daily and any change in dietary habits or activity was monitored very closely. At each week one rabbit from each group is randomly taken and sacrificed by using over dose lethal intravenous administration of sodium pentobarbital (100 mg/kg)[17]. Sodium pentobarbital is the drug of choice because it acts fast and effective [18], painless and without prior stress to the animals [19].

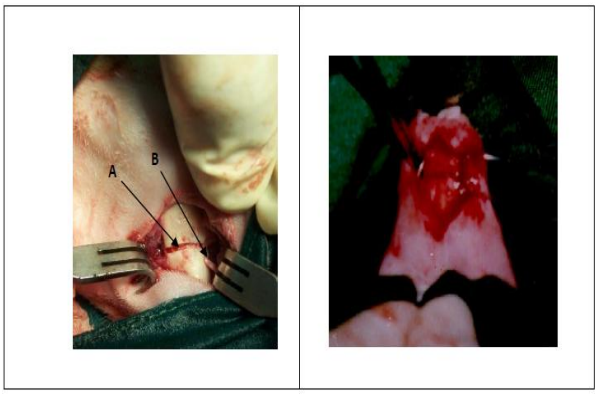

Fig. 1: A= osteotomy site, B= mental nerve, Fig. 2: Passing stainless steel wire (gauge 0.5 mm) through the two holes on each side of the fractured mandible bone.

Mandibular bone preparation for evaluations were explanted immediately after the animals were sacrificed. All the rabbit mandibles from the three groups of study were subjected to tissue processing and sectioning. Block sections of the surgical sites were fixed in 10% neutral buffered formalin for 7 days, fixed specimens followed by decalcification and dehydration in 5% formic acid, at 4°C for 14 days and finally embedded in paraffin and sectioned at 5 µm thickness. Thick sections were cut along these and stained with hematoxylin-eosin (H–E) and Masson's trichrome staining were performed separately on consecutive tissue sections for histologic analysis. After microscopic examination, a photographic system Olympus Model (PM-10 AD, Japan) with lens (4 × 5) FK (NFK) was used to investigate the histopathological pictures on the slides of interest.

RESULTS

Histopathological examination was done in the Department of Oral Pathology, College of Dentistry, Baghdad University. Two independent histopathologist were asked to give their conclusions about each sample by the use of double-blind technique. Karl-Pearson coefficient of correlation was calculated (r=0.9), which indicate a high agreement between the two pathologists.

Keys of the histopathological slides

Key	Details
A	Acute inflammatory cells
B	Bone trabecule
C	Collagen fibers
E	Empty lacune
F	Fibers tissue
O	Osteoid formation

At week 1 group A rich protein an acute inflammatory reaction was presented, showing acute inflammatory cells in the bone marrow with fibrin deposition. The bone of the margin of the fracture shows the absence of the osteocyte cells (Fig. 3). Group B control group, bone showed a mild acute inflammatory reaction in the bone marrow spaces with small number of acute inflammatory cells. The margin of the bone at the fractured line shows irregular deformity (Fig. 4). The group C restricted protein, bone shows R.B.Cs extra vasation. In the bone marrow area there is a small number of acute inflammatory cells. Mostly neutrophils scattered within soft tissue of the bone marrow (Fig. 5). At week 2 group A rich protein, the fractured gap is filled by soft tissue showing several areas of newly form osteoid tissue with the beginning of calcification. The restricted group C, the fractured gap is filled by soft tissue composed of collagen fibers and fibroblast, osteoblast cells are seen at the periphery of the bone trabecule on the fractured line. There are number of evidence of osteoid tissue formation at this time.The control group B, the fractured gap is filled by fibrous tissue showing

some areas of osteoid tissue formation. At week 3 group A rich protein, the fractured gap is filled by bone trabecule with some areas of fibrous connective tissue. The restricted group C, the area of fractured gap shows dense fibrous connective tissue with areas of newly formed osteoid tissue with evidence of calcification. The control group B, the gap is filled by fibrous connective tissue. There is an evidence of formation of bone trabecule in the gap area connecting between the two pieces of the fractured bone. At week 4 group A rich protein, the fractured gap is filled by dense calcification bone with few osteocyte cells in the bone trabecule. The restricted group C, the fractured gap is filled by soft tissue with bone trabecule formation.The control group B, The slide shows complete filling of the fractured gap by bone trabecule which present at the margins near the fractured line immature bone trabecule. At week 5 group A rich protein, there is a complete formation of the bone in the fracture indicated by the gap and the bone trabecule that is connecting the two ends of the fractured bone, and the newly formed bone is completely matured (Fig.6). The restricted group C, the bone trabecule in the fractured gap is more evident and mature but still there is some soft tissue and there is no complete union between the two ends of the fractured bone. The control group B, the fractured gap is filled by bone trabecule and there is complete union between the two ends of the fractured bone. At week 6 group A rich protein, the fractured gap is filled by fully mature bone with large bone trabecule. The newly formed bone is connecting between the two ends of the fractured bone.The restricted group C, the bone trabecule filling the gap between the two fractured ends is well developed but still there are large areas of the soft tissue present between the bone trabecule. The control group B, the histopathological appearance of this slide is similar to that seen in the previous week (Fig. 7). At week 7 group A rich protein, the histopathological appearance of this slide is similar to the histopathological picture of the previous week. The restricted group C, the fractured gap is filled by fully mature bone trabecule connecting between the two ends of the bone. The control group B, the histopathological appearance of this slide approximately similar to the previous week with well formed trabecule and complete healing of the fractured bone. At week 8 group A rich protein, the histopathological appearance of this slide is similar to the histopathological picture of the previous week. The restricted group C, the fractured gap is filled by fully mature bone trabecule connecting between the two ends of the bone (Fig. 8). The control group B, the histopathological appearance of this slide approximately similar to the previous week with well formed trabecule and complete healing of the fractured bone.

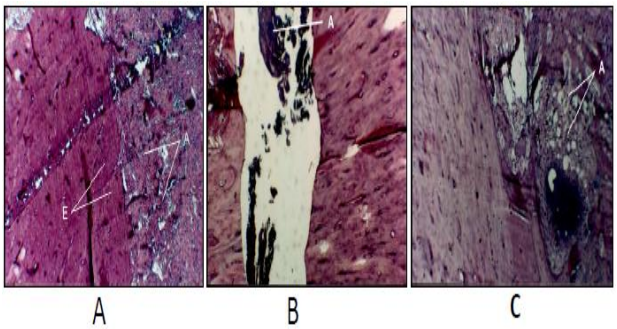

Fig. 3: Rich protein group A (week 1) after surgery, Fig. 4: Control group B (week 1) after surgery, Fig.5: Restricted protein group C (week 1) after surgery

STATISTICAL ANALYSIS

We noticed that the inflammatory degree can be put on a scale of eight (8) grades. The coding procedure of the inflammatory degree depends on the progress of the animal condition during the period of treatment as well as the sign obtained through the investigation of the histopathological slides.

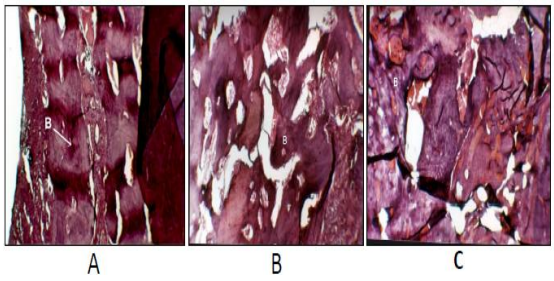

Fig. 6: Rich protein group A (week 5) after surgery, Fig. 7: Control group B (Week 7) after surgery, Fig. 8: Restricted protein group C (Week 8) after surgery

The slides of the rich protein group were given the numbers 1-8 according to the weeks of observations. The slides of the other two groups were compared and given codes 1-8 according to the sign(s) found on the slides. Table 1 shows the coding criteria for the inflammatory degree.

Table 1: Coding criteria for the stages of healing degree.

degree	Signs of healing
1	Infiltration (slight, mild, severe)
2	Osteoid tissue (slight, mild, sever)
3	Bone trabecule (slight, mild, severe)
4	Calcification (slight, mild, severe)
5	Bone formation
6	Maturation (slight)
7	Maturation (mild)
8	Maturation (full)

The healing process was first put on a scale of three (3) grades according to the previous definition. The practical findings of the information regarding such variable suggests that the last stage of the healing process should be subdivided into two categories namely; 3A and 3B. Since such type of data would not be acceptable for the purposes of statistical analysis, the two categories were given the code of 3 and 4 respectively instead of the previous coding (3A and 3B). The data are shown on table 2.

INFERENTIAL STATISTICS

The one-way analysis of variance was used twice in this study. First it was used to test whether the mean inflammatory degree is significantly different according to the three groups of rabbits (Table 3 and Fig. 9). Second, it was used to test whether the mean healing process is significantly different according to the three groups of the rabbits (Table 4 and Fig. 10). With reference to the inflammatory degree, the one-way analysis of variance shows that mean inflammatory degree of the 'Rich Protein' group is significantly greater than that of the remaining two groups. This is of course can be interpreted in terms of the direct effect of the protein. This figure clearly shows the delay time in the inflammatory degree for the restricted group of rabbits, whereas the other two groups revealed a rapid body response to the fracture reflected by the mean inflammatory degree. The one-way analysis of variance was also used to investigate the differences between the means of healing stages among the three groups of rabbits. The results of this test showed a significant difference between the mean healing stage of the 'Rich Protein' group and the mean healing stage of the 'Restricted' group. The results that are shown in table 4 and figure 10, indicated that there is a remarkable difference between the mean healing stage of the 'Rich Protein' and 'Control' groups in addition to the significant difference between the mean healing stage of the 'Rich Protein' group in comparison with 'Restricted' group. This figure shows how rapid is the approaching of the last stage of healing in the "Rich Protein' group A of rabbits in comparison with other two groups of rabbits. The figure indicates the dramatically difference between the mean of the healing process for the 'Rich Protein' A in comparison with that of the 'Restricted' group, C which again shows the influence of the protein on the healing process.

Table 2: Histopathological findings and healing process for the sample units over the period of the experiment

S. No.	Sample' type and code	Duration (Week)	Inflammatory degree	Stage of healing	Censoring
1	Rich Protein (1)	1	4	1	0
2	Restricted (2)	1	1	1	0
3	Control (3)	1	2	1	0
4	Rich Protein (1)	2	4	2	1
5	Restricted (2)	2	2	1	0
6	Control (3)	2	3	1	0
7	Rich Protein (1)	3	5	2	1
8	Restricted (2)	3	2	1	0
9	Control (3)	3	4	1	0
10	Rich Protein (1)	4	6	3	1
11	Restricted (2)	4	3	1	0
12	Control (3)	4	5	1	0
13	Rich Protein (1)	5	7	3	1
14	Restricted (2)	5	4	1	0
15	Control (3)	5	6	2	1
16	Rich Protein (1)	6	8	4	1
17	Restricted (2)	6	5	2	1
18	Control (3)	6	6	3	1
19	Rich Protein (1)	7	8	4	1
20	Restricted (2)	7	6	3	1
21	Control (3)	7	6	3	1
22	Rich Protein (1)	8	8	4	1
23	Restricted (2)	8	7	3	1
24	Control (3)	8	7	4	1

Table 3: Analysis of variance table for the inflammatory degree by the three groups of rabbits

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-test	P-value
Groups of rabbits	2	25.08	12.54	3.57	0.046
Error	21	73.87	3.52		
Total	23	98.96			

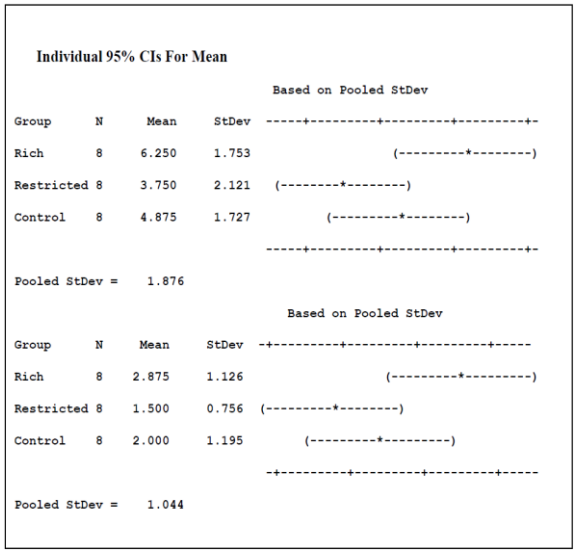

Fig. 9, 10: The 95% confidence intervals for the differences between the means of the animal groups with regard to the inflammation and healing stage codes respectively.

The relation between the inflammatory stage and healing stage can be concluded then through the results of the one-way analysis of variance that suggest the existence of positive relationship between the two variables. This also means that the healing process can be hold as a function to the inflammatory degree. It is of interest to investigate the healing time at which the healing process approaches the third stage despite the duration before the perfect healing. In this contrast the survival analysis was performed and the Kaplan-Meier estimates were used to calculate the estimated probabilities to approach the third stage of the healing process. The mean time before approaching the perfect healing of the bone was also calculated under the assumption of normal probability distribution. Since the last results obtained in this research work were recorded after the seventh week of the experiment, we will use these records as the data obtained for the beginning of the eighth week.

Table 4: Analysis of variance table for the healing stage by the three groups of rabbits.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-test	P-value
Groups of rabbits	2	7.75	3.87	3.56	0.047
Error	21	22.88	1.09		
Total	23	30.63			

This procedure was very necessary to put a deadline to the late category of the third stage in this research. Table 5, shows the results obtained by the use of survival analysis for the data of this research. It is clear from the results stated in table 4 that the 'Rich Protein' group A arrives to an earlier complete healing in comparison with the other two groups. The 'Control' B and 'Restricted' group C, however, are also different due to the significant differences in the survival estimates obtained by the Kaplan-Meier as stated in the table 5. Fig. 11, clearly shows the difference in the healing process regarding the three groups of rabbits according to the survival estimates mentioned in table 5.

Table 5: Kaplan-Meier estimates, and the mean time and standard deviation before approaching the perfect healing.

Group	Mean time (Weeks)	(SD) Weeks	Weeks	Kaplan-Meier
Rich			4	83.33
Protein A	6.51	1.69	5	66.67
			6	50.00
			7	33.33
			8	16.67
			7	66.67
Restricted	8.01	0.81	8	33.33
C			9	3.33
			6	75.00
Control B	7.51	1.11	7	50.00
			8	25.00
			9	2.50

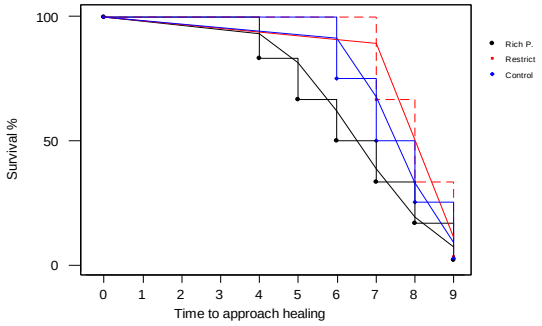

Fig. 11: The Survival plot to the time of healing process for the three groups of rabbits

DISCUSSION

Morphological features and biochemical metabolism of the mandible are negatively affected by protein-energy malnutrition [20]. This study aimed to investigate how this effect on the healing time of fractured mandibular bone. For this reason the three groups of rabbits mentioned earlier in this study were considered. Approximately 5% to 20% of fractures have delayed or impaired healing for various reasons [21]. This research focuse on the delay time for the healing of mandibular bone fracture in this study. Since the healing process is affected by many factors [22, 23, 24, 25], therefore, the inflammatory degree and the healing stage are carefully handled and treated in this study because they involved most of the effects explained by the nutrition, age, as well as general health status of animal body. The results of the one-way analysis of variance revealed that the 'Restricted' group of rabbits shows an inflammatory delay response. This result is in agreement with previous studies [26, 27]. The results of present study clearly shows the effect of nutrition on the healing of mandibular fracture. Therefore, one might conclude that the type of diet is a very important factor in the process of bone healing. The result of the one-way analysis of variance regarding the inflammatory degree with respect to the restricted protein group can be explained in terms of the signs and symptoms that are found to be accompanied by the protein deficiency [28]. Protein is present in all cells of the body and therefore the insufficient intake of nitrogen-containing food (protein) to maintain a nitrogen balance or nitrogen equilibrium, will impair body response toward any abnormal regenerative events such as wounds healing, fractures, etc. The negative impact of involuntary weight loss and protein-energy malnutrition (PEM) on local wound healing has long been recognized [29]. It has been also proved that morphological features and biomechanical function of the mandible with regard to protein-energy malnutrition as indicated by body size, the mandibular base length, height and area were significantly smaller in malnourished than control rats [30]. Generally immunological response considered important for the wound healing mechanism success and this response depend on protein intake so restricted protein intake mean weaken immune response.Such effect can be used to explain why the restricted protein group showed a delay inflammation degree. The result indicates that the 'Rich Protein' group A of rabbits converge the late of the third healing stage significantly ($p<0.05$) earlier than the other two groups of animals. This is also indicating the direct effect of nutrition on the healing process of mandibular bone fracture. Although, the 'Control' group B of rabbits shows a remarkable earlier convergence to the the third stage of mandibular bone healing in comparison with the 'Restricted' group C of rabbits, but they are still delayed in comparison with the 'Rich Protein' group A of rabbits. This clearly indicated how important the presence of high protein nutrition in making such difference. In present study we found that the histology of the bone healing among the three groups is demonstrated by healing at 8 weeks in group C and 6 weeks in group B equal to healing at 4 to 5 weeks for group A. (Fig. 6,7 and 8). The findings regarding the control group is in agreement with Oversen, et al., [1991][30]. Oversen who stated that addition of nutritional supplements to regular meal programs provides extra nutrition that may assist in wound healing, recovery from infection, or improvement in general nutritional status. In these settings, Oversen, et al, [1991][30] were referring to supplements of an energy-rich beverage containing carbohydrate, protein, fat and a complement of vitamins and minerals. Other authors also found that the clinical outcomes were significantly improved for those hip fracture patients receiving a protein-containing supplement rather than a supplement that did not contain protein [31]. The protein supplement in this study increased usual protein intake by more than 60%. Thus, it appears that patients who have hip fractures and are frequently malnourished upon hospitalization benefit from a liquid nutrition supplement containing protein. Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as ferrous iron and vitamin C. Protein is one of the most important nutrient factors affecting wound healing [32]. A deficiency of protein can impair capillary formation, fibroblast proliferation, proteoglycan synthesis, collagen synthesis, and wound remodeling, also affects the immune system, with resultant decreased leukocyte phagocytosis and increased susceptibility to infection [33]. Vitamin C has many roles in wound healing, and a deficiency in this vitamin has multiple effects on tissue repair. Vitamin C deficiencies result in impaired healing, and have been linked to decreased collagen synthesis and fibroblast proliferation, decreased angiogenesis, and increased capillary fragility. vitamin C deficiency leads to an impaired immune response and increased susceptibility to wound infection so impaired wound healing results from deficiencies in any of these factors [34,35]. This result is also emphasized by Efthimious, et al [1988][32], who examined the effectiveness of a 3-month liquid nutrition supplementation trial in out-patients with COPD. It is clear from many other studies that malnutrition and specific nutrient deficiencies compromise the body's wound healing process while the overall effects of poor nutritional intake increase an individual's susceptibility to pressure sore development [36, 37, 38]. Therefore, an adequate energy and protein intake are essential in order to prevent protein energy malnutrition. Since, malnutrition can result in delayed wound healing, early nutritional intervention may influence the rate of healing [39]. It was also shown that if patients are unable to meet their nutritional requirements, oral nutritional supplementation is far less than the costs associated with poor wound healing seen in undernourished patients [40, 41]. Dietitians should provide a high-energy, high-protein diet for patients to improve their dietary intake and nutritional status [42]. With regard to the phases of bone healing pointed out by Wehrli, [2003][43], it was also stated that their duration depends on age, health, immobilization, complexity of fracture and nutritional status. They affect the inflammatory,reparative and the remodeling phase of healing. Moreover, the positive relationship between the healing stage and the inflammatory degree is indicating how robust is the mechanism of such a relation that can be used to assess a mathematical function explaining variability in the healing process [44]. In the survival analysis the difference in the mean time required to converge to the third stage of mandibular bone healing is very clear. The difference between the 'Rich Protein' group and the

other two groups is almost two weeks on the average bases. We have to bear in mind that converging to the third stage should not necessarily be interpreted as that the bone has perfectly healed (acquiring its original shape and rigidity). In this contrast, we may state that it may take a long time before approaching perfect healing, and sometimes this can be never approached, particularly if we know that approximately 5% to 20% of fractures have delayed or impaired healing [45]. It is of interest to mention the effect of sex, race, and other anatomical factors on the process of bone healing in general. In this study all the rabbits were considered to be from the same age, sex and racial group.

This consideration will eliminate the effect of such factors on the healing process. Accordingly, the effect will only restrict to the diet. The production of a better and stronger healing bone has attracted the interest of many investigators in the past [44,45]. Numerous substances have been used to increase both the strength and rate of production of fracture callus [45, 46]. The present study confirmed other studies for advantages of using immature rabbit experimental model have a greater regenerative potential after five weeks time review of bone healing all the animals had histological study demonstrated that healing process in advanced stage [47]. Animal models not always give adequate parameters for humans which includes mouse, horse, rat, cat, dog, sheep, chicken, pigeon, primates, rabbits and less common animals [48,49]. Prolo et al. [1982] stated in his study that humans do not react the same as other animals to wounds healing in calvaria and he justify that of a poorer blood supply in the animal calvaria and some deficiency of bone marrow mean harvesian bone system different [46]. Rats and mice have a healing process that is different from humans, since they haven't a Harvesian bone system and although little is known about the importance of this anatomical difference between rodents and humans [50], preventing studies aiming to evaluate the form, function or material and biomechanical properties of Harvesian system may not extend in the same manner to humans [48,51]. These make bone repair in these animals different from that is seen in human beings [52]. For this reasons every model of research has advantages and disadvantages, and the researcher should be wise to retrieve from the literature the better fitting to the proposed study method. Large animals such as mammals, dogs and primates request expensive costs for their use [48]. It is important that a number large enough of animals is used, in order to reach a statistic significance [48,53]. Twenty, thirty, or forty large sized animals in a research can be very expensive, and handling them can be very difficult for a regular investigator [48]. Rabbits considered the most popular animal models in health science, most of the researchers are being used in approximately 35% of musculoskeletal research studies [54]. Their size is small enough to allow the use of mode, convenient in that it reaches skeletal maturity shortly after sexual maturity at around 6 months of age [55].

CONCLUSIONS

Throughout the different practical aspects of this study, we come up with the following conclusions:

1- Inflammatory degree for the 'Rich Protein' group of rabbits is rapidly progressed, whereas its progress was noticed to be slow down for the other two groups. Consequently the healing process is positively affected by this result, due to the robust positive relationship between the two criteria (inflammatory degree and healing process) that is clearly through the indices of the table 2.

2- The late stage of the healing process does not necessarily mean that bone acquired its normal shape and rigidity. It may take long time before approaching such a stage, or sometimes this stage never be approached.

3- The average time (in weeks) required to approach the late stage of healing process in the 'Rich Protein' group is found to be 6.51 with standard deviation equals to 1.69. This average, if we take the standard deviation in consideration, will almost exceeds the average time in the remaining two groups by 1.5-2 weeks. The 'Restricted' group required a long time to approach the late stage of healing process. 4- The healing process is found to play the role response variable (dependent variable) for the inflammatory degree (independent variable).

5- Deformation is only noticed in the first sample of the 'Restricted' group of rabbits. This of course reflects the bad effect of malnutrition on the healing process.

ACKNOWLEDGMENT

The authors would like to thank School of Dental Sciences, Universiti Sains Malaysia (USM) and University of Baghdad- Iraq for the financial support.

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