Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Effect of autologous platelet-rich plasma versus platelet-rich fibrin on the second intension wound healing in dogs through higher regeneration capacity and modulation of inflammatory cytokines

Olla A. Khalifa[1], Abdelhaleem H. Elkasapy[2*], Eman A. Sallam[3], Adel M. Alakraa, Yasmin M. Marei[4] and Liza S. Mohammed[5]

[1] *Genetics and Genetic Engineering, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2] *Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Benha University, Egypt.*

[3] *Animal and Poultry Production, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Egypt.*

[4] *Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Benha University, Egypt.*

[5] *Veterinary Economics and Farm Management, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Egypt.*

ARTICLE INFO

Keywords

Dogs
Gene expression
PRF
PRP
Wound

Received 25/08/2021
Accepted 03/09/2021
Available On-Line 01/10/2021

ABSTRACT

Platelet rich plasma (PRP) and platelet rich fibrin (PRF) are considered excellent concentrated sources of growth factors (PDGF, TGF-β, VEGF, bFGF) and cytokines fundamental for wound healing. The aim of this study was to evaluate and compare the effect of PRP and PRF on the second intention wound healing through clinical vision, histopathological examination, and changes in interleukin 10 (IL10) and transforming growth factor-β (TGF-β) genes expression. Fifteen adult dogs were used in this study: induction of a 3 cm diameter total thickness cutaneous injury at the right chest region. The animals were divided equally into three groups, and the wounds were treated twice weekly for three successive weeks. The first group (Group A) received only normal saline (control group). The second group was treated by PRP (group B). The third group received PRF treatment (group C). Clinical evaluation, molecular studies of IL10 and TGF-β gene expression, and histopathological examination were used to demonstrate the difference between the three treatment regimens. Results showed a non-significant negative correlation between weight loss and wound healing rate (WHR%), but a significantly high positive correlation between treatment cost either by PRP or PRF with IL10 (0.79*) and WHR% (wound healing rate) (0.994**). The IL10 significantly increased in PRP group, while TGF-b significantly increased in the PRF group. This study concluded that the PRP and PRF exhibited higher regeneration capacity and accelerated the quality of wound healing.

1. INTRODUCTION

In the second intention, wound healing represents a significant problem in the veterinary practice, as it is more susceptible to infection with multidrug-resistant strains of pathogenic microorganisms and dryness. The healing process begins directly from the boundaries of the wounds after the occurrence of the skin injury. The blood clot also acts as a bridge for cell migration. The blood plug replaced by granulation tissue three to five days later ushered in a start of re-epithelization (Farghali et al., 2017).

Wound healing is a process that includes four overlapping remodeling (Singer and Clark, 1999), involving numerous inflammatory mediators, immune cells, extracellular matrix (ECM), and parenchymal cells (Bielefeld et al., 2013). It begins with the homeostasis/inflammatory response, platelet aggregation, and production of numerous cytokines. In the proliferation process, migration and proliferation of keratinocytes, endothelial cells, and fibroblasts occurred. The recovery of the dermis occurs after invading and proliferating by fibroblasts, which is considered significantly crucial for wound healing (Singer and Clark, 1999). The tissue remodeling phase includes the newly formed capillaries and collagen reorganization; Collagen is produced by fibroblasts and plays an essential role in each phase (Kallis and Friedman, 2018).

Platelets exhibited effective antimicrobial activity, which improved the infected wound healing, re-epithelization, and granulation tissue formation (Bielecki et al., 2007). Platelets are a great source of wound healing agents as a platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and platelet-derived angiogenesis factor (PDAF) (Nikolidakis and Jansen, 2008), so increasing their concentrations in the affected area enhance, reinforce and accelerate the healing process (Mishra et al., 2009). PRP

* Correspondence to: abdelhaleem_elkasapy@yahoo.com

(platelet-rich plasma) provides two of the three components (i.e., growth factors and scaffold) necessary to support proper tissue regeneration and improvement significantly in the healing of complex wounds (Villela and Santos 2010). PRF (platelet-rich fibrin) belongs to a new generation of platelet concentrates, simplified processing, and biochemical blood handling. PRF contains concentrated growth factors (PDGF,TGF-β,VEGF and basic fibroblast growth factor (bFGF)) (Kimura et al. 2005; Dohan et al. 2006).

Interleukin-10 (IL-10) is a homodimeric cytokine produced by various cell types, including T cells, monocytes, and macrophages. In the skin, keratinocytes produce IL-10 after injury (An et al., 2010), activate macrophage/ monocyte functions, decrease pro-inflammatory cytokine production and regulate fibrogenic cytokines, such as (TGF-b) as a part of its role in the regulation of tissue remodeling (Moore et al., 2001; Fortunato et al., 1998). Wounds infiltrated by M2-activated macrophages secreted TGF-β (Sato et al., 2010; Brancato and Albina, 2011), which stimulates migration and proliferation of fibroblasts at the wound site (Werner and Grose, 2003). TGF-β have both anti-inflammatory activity (Shull et al., 1992; Kulkarni et al., 1993), and pro-inflammatory effects (Gilbert et al., 2016), with regard that pro-inflammatory effects are predominant (Wang et al., 2006; Han et al., 2012). It has been reported that persistent inflammatory infiltrates in chronic ulcers contribute to TGF-β levels (Finnson et al., 2013; Gilbert et al., 2016). Our work aimed to evaluate and compare PRP and PRF's effect on the second intention wound healing and the role of IL10 and TGF-β gene expression. The evaluation and comparison between the three treatment regimens were carried out through clinical vision, real-time quantitative PCR, and histopathological examination.

2. MATERIAL AND METHODS

2.1.Experimental Animals

The study was conducted on fifteen mongrel dogs aged 2-3 years and weighing 12-14 Kg. The animals were kept in separate kennel under slandered environmental conditions (24± 1c, 55±5 % humidity band a12h light/dark cycle). The animals were given free access to water and were given maintenance ration twice daily. Under the influence of general anesthesia (Waelbers et al., 2009), an acute full-thickness skin wound of 3 cm diameter was made on the right side of the vertebral column in the chest area.

All animal experiment processes were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine Benha University (BUFVTM 05-08-21). It was strictly designed under the consideration of animal welfare

2.2. Study Groups

The dogs were divided into three groups; five dogs each, the wounds of all groups treated twice weekly for three successive weeks. Group A (control group): the wounds were subjected only to normal saline. Group B (PRP treatment): the wounds were treated by autologous PRP, where the wound's edges were well covered, as was the wound itself. Group C (PRF treatment): the wounds were treated by autologous PRF by the same technique adopted in the second group. Then leave the wounds of all groups to heal with the second intention.

2.3.Preparation of PRP

The blood obtained from the dogs was placed into vacuum blood collection tubes containing citrate-phosphate-dextrose solution and centrifuged for 10 min at 1500 rpm. After centrifugation, three layers were obtained. The plasma layer at the top was collected in another centrifugation tube and subjected to second centrifugation for 10 min at 3000 rpm, after which two layers were obtained; the upper layer was platelet-poor plasma (PPP) the lower was PRP (Esat et al., 2016). This final form of liquid PRP was applied to the wounds of the dogs.

2.4. Preparation of PRF

For each dog, the blood sample (one tube of 15 ml each) was obtained from a jugular vein. The blood samples were taken without anticoagulant in dry glass tubes and immediately centrifuged at 3,000 rpm for 10 minutes with a specific table centrifuge. After centrifugation, the PRF clot was removed from the tube using sterile tweezers, separated from the RBC base using clean scissors, and placed in a clean cup (Dohan Ehrenfest et al., 2010).

2.5. Evaluation of wound contraction rates

Clinical evaluation was achieved through digital photographs taken from wounds in the presence of a ruler for measuring wound healing rate at days 0, 7, 14, and 21 after injury. The wound healing rate in control, PRP, and PRF-treated wounds was calculated according to the following equations:

WHR% = (iWA- fWA)/iWA x 100 (Eldebany et al. 2020).

WHR is the wound healing rate, iWA is the initial wound area and fWA is the final wound area

2.6. Real-time quantitative PCR

The skin section was taken immediately from anesthetized animals and placed in Cryo tubes and stored in RNA later solution (by ten µL per 1 mg of tissue) (Qiagen-GmbH, Germany) at-80°C. Total RNA was isolated using Easy Red TM kit (Intron Biotechnology, Korea) according to the manufacturer's instructions; it was eluted in nuclease-free water, purity quantified spectrophotometrically at 260 and 280 nm, and kept at −80 °C until use. RPS-5 normalized transcript quantities as a housekeeping gene. Ten µL RNA (2 µg) from each sample were taken for the Synthesis of cDNA using RT2First Strand Kit (Qiagen-GmbH, Germany) according to the manufacturer's instruction. Quantitative Real-Time PCR amplifications were performed with RT2 SYBR Green master mix (Qiagen-GmbH, Germany), in the thermocycler Real-time PCR (Applied Biosystem 7500 Fast Real-time PCR, USA), under the following cycle conditions: 95°C for 10 min followed by 40 cycles of 95°C for 15 sec then 60°C for 1 min. The fold-change for each gene calculated using the formula: 2^ (-ΔΔCT) (Livak and Schmittgen, 2001). The p values are calculated based on a Student's t-test of the replicate 2^(-Delta Ct) values for each gene in the control group and treatment groups and p values less than 0.05. The following primers (Invitrogen, Thermo Fisher Scientific, USA) were used for cDNA amplification (Table 1).

Table 1. List of primers sequence.

Target gene		Primers	Ref.
RPS-5	F	TCACTGGTGAGAACCCCCT	(Brinkhof et al., 2006).
	R	CCTGATTCACACGGCGTAG	
IL-10	F	CCCGGGCTGAGAACCACGAC	(Veenhof et al., 2010).
	R	AAATGCGCTCTTCACCTGCTCCAC	
TGF-β	F	CAAGGATCTGGGCTGGAAGTGGA	(Spee et al., 2005).
	R	CCAGGACCTTGCTGTACTGCGTGT	

RPS-5,ribosomal protein S5; IL-10, Interleukin 10;TGF- β, Transforming growth factor beta

2.7. Histopathological Evaluation

Specimens for histopathological evaluation were obtained on day 21 after surgery; the specimens were fixed in 10% neutral buffered formalin. After proper fixation, the skin tissues were gradually dehydrated, cleared and embedded in paraffin. Then, a microtome was used to cut the tissues into 5-µm-thick sections. Paraffin sections were stained by Hematoxylin and Eosin (H&E) for routine light microscopic examination and Masson's trichrome stain to detect connective tissue maturation. The depth and organization of the green color can differentiate the maturity of collagen fibers (Bancroft and Gamble, 2008). The degree of collagen production, cellular infiltration, neovascularization, and re-epithelialization process, including the thickness of epithelium over the wound, is the wound healing parameters. Histopathological evaluation of these parameters allowed the detection of differences between control and treated groups.

2.8. Productive and economic measures

Bodyweight was recorded individually for each dog in each group at the beginning and the end of the experiment. Treatment cost was defined as the cost of inputs used to treat disease (Bennett and IJpelaar, 2005): The Control group receives only dressing (cost of dressing per day * the number of days for each animal. PRP receives dressing + PRP (PRP cost per day * the number of days) for each animal. PRF receive dressing + PRF (PRF cost per day * the number of days) for each animal.

2.9. Statistical analysis:

The present investigation's collected data are subjected to statistical analysis using one-way analysis of variance (ANOVA) to determine wound dimensions, means of different variables as TGF, IL10, body weight changes, and treatment cost. A correlation matrix was done to determine the relationship between variables as body weight changes and treatment cost on TGF, IL10, and WHR % Linear regression: The regression test was used to determine the best way of the correlations between WHR%, TGF, and IL 10 (dependent variable) with loss of weight and treatment cost (independent variable). The functions' model was the logarithmic model. (T-test) used to determine the significance of each relationship. Also, the adjusted coefficient of determination (R^{-2}) was calculated to assess the degree of each relationship between dependent and independent variables (SPSS, 2007).

3. RESULTS

3.1. Clinical findings

The control group's wounds (group A) exhibited sepsis and elevated center in 2 of the induced injury, while the other three showed dryness, thin fissuring of the blood blog during the 2nd week. The PRP and PRF showed no sepsis and smooth surface and rapid healing with no significant difference in wound healing between them, but the two groups showed a substantial decrease between them and the control group's animals. The criteria of wound healing in groups B and C run in speed more than the control group (Table 2). The PRP and PRF groups were significantly ($p < 0.05$) lower than the control group at 1st to 3rd weeks

3.2. Expression of IL10 & TGF-β in canine treated with PRP & PRF

Quantitative RT-PCR was performed to measure mRNA expression of IL10 & TGF-β in dogs with second intension wound followed by treatment with PRP & PRF to assess healing. Comparing with control group there was a significant up-regulation in the expression of IL 10 in experimental groups (PRP and PRF) with a substantial increase in expression of IL10 in the PRP group than the PRF group ($P<0.05$) (Fig 1 a). Also, there was significant up-regulation in TGF-b expression in the PRF group than in the PRP group ($P > 0.05$) (Fig 1 b).

Table 2. Clinical evaluation (wound dimension and wound healing rate percent of the three groups.

	1st week	2nd week	3rd week
Wound dimensions			
Control	26.83 ±0.166[a]	21.33 ±0.33[a]	15.33 ±0.33[a]
PRP	22 .5±0.289[b]	10.33 ±0.33[b]	0.7 ±0.166[c]
PRF	23.5 ±0.289[b]	11.67 ±0.33[b]	2.33 ±0.33[b]
Wound healing rate %			
Control	10.7[c]	29.0[c]	49.0[c]
PRP	25.0[a]	65.7[a]	97.7[a]
PRF	21.7[b]	61.3[b]	92.3[b]

Platelet-rich plasma (PRP), platelet-rich fibrin (PRF). Values are mean ± standard error (SEM). The mean differences between the values bearing different superscript letters within the same column are statistically significant ($p < 0.05$).

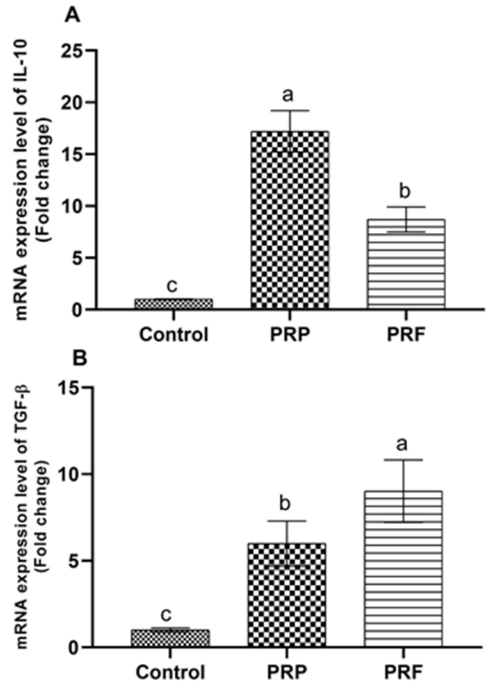

Figure 1. Relative mRNA expression for (a) IL 10 and (b) TGF-β were determined by comparison with the value of the control group and treated groups for each dog normalized against RPS-5 as a housekeeping gene. Values are mean ± standard error (SEM). The mean differences between the values bearing different superscript letters within the same column are statistically significant ($p < 0.05$).

3.3. Histopathological findings

Histopathological results of wounds on Day 21st after surgery are corresponding to the final stage of wound healing. The re-epithelialization process, in all groups, was occurring, notably extending to the wound surface, and covered by scab tissue or keratin. Occasionally, the proliferated epidermal epithelium in the control group showed focal areas of incomplete re-epithelialization, composed of granulation tissue admixed with variable numbers of lymphocytes, few macrophages surrounded by one or several layers of keratinocytes (Fig.2A). The PRP-treated cases showed higher re-epithelialization levels in the healed wounds with rete ridges formation in underlying granulation tissue (Fig.2B). The PRF-treated group showed complete re-epithelialization; however, the thickness of epithelium over the wound was lower when compared with that of the wounds treated with PRP (Fig.2C), which indicated that re-epithelialization in this group was much slower than that in the PRP-treated group.

The rate of collagen production was demonstrated in the dermis. The PRP-treated cases had the highest level of collagen deposition (Fig.3B). Meanwhile, the PRF-treated wounds had moderate collagen deposition (Fig.3C) compared with the control group, which had granulation tissue formation with minimal collagen deposition (Fig.3A) in the dermis of wounds. These results were confirmed by the Masson's trichrome staining of collagen, where the green color of the PRP-treated wounds was the most intense, indicating highly mature collagen fibers (Fig. 4). However, the control group presented the highest number of new blood vessels to form granulation tissue characterized by fibroblasts and collagen perpendicular to new blood vessels (Fig. 3A). The number of new vessels of the control cases reduced in PRP and PRF-treated wounds (Fig. 5). The dermis granulation tissue was infiltrated by small aggregates of inflammatory cells, mainly lymphocytes and macrophages. The number of inflammatory cells in PRP and PRF treated groups was higher than observed in the control group (Fig. 5)

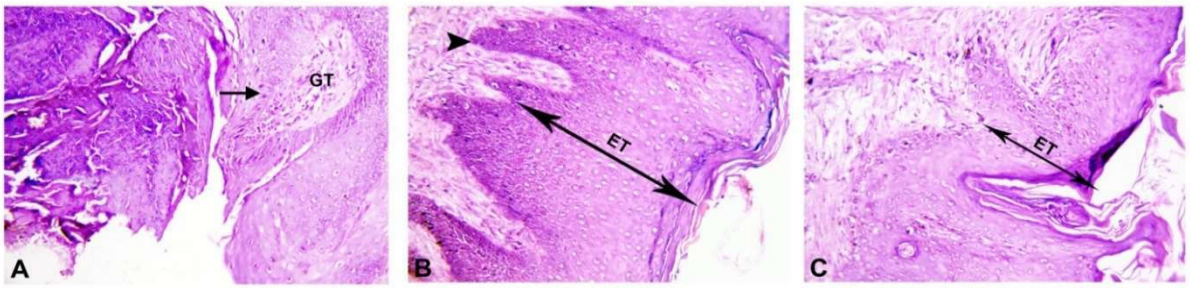

Figure 2. Re-epithelialization of wound healing in dog on day 21 after-surgery: (A) Control group showing a focal area of incomplete re-epithelialization, composed of granulation tissue (GT) admixed with inflammatory cells and surrounded by several layers of keratinocytes (arrow). (B) PRP-treated group showing higher re-epithelialization level (ET) with rete ridges formation (arrowhead). (C) PRF-treated group showing complete re-epithelialization with lower epithelium thickness (ET) (X20; H&E stain).

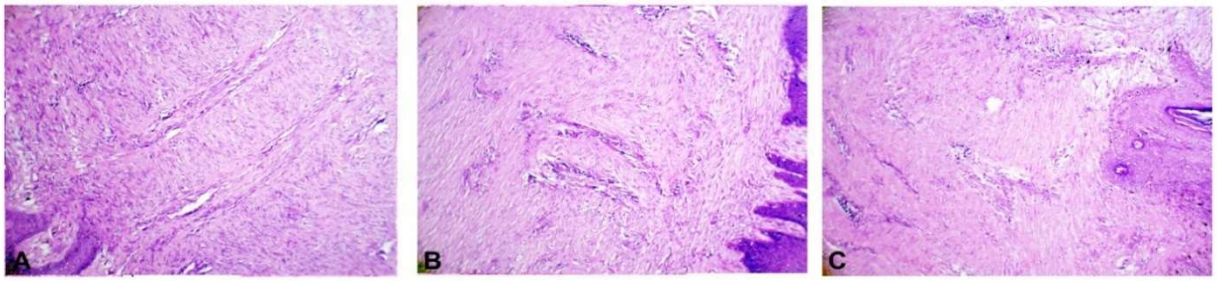

Figure 3. Collagen production in the dermis of wound healing in dog on day 21 after -surgery: (A) Control group showing minimal collagen deposition. Note fibroblasts perpendicular to new blood vessels (B) PRP-treated group showing a high collagen deposition level. (C) PRF-treated group showing moderate collagen deposition (X10, H&E stain).

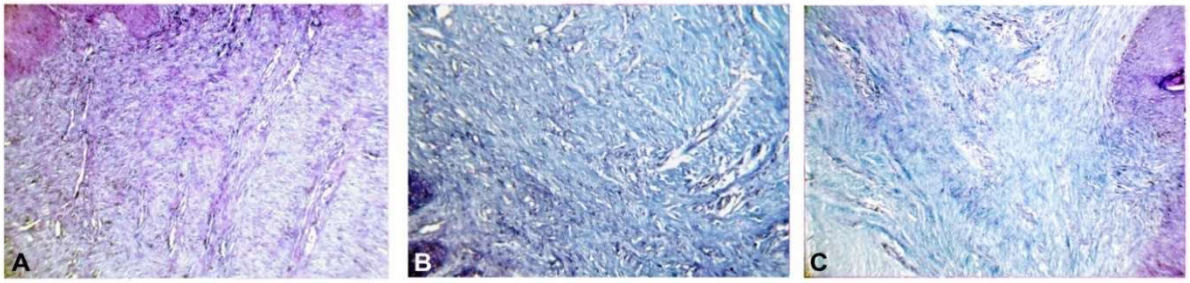

Figure 4. Masson's Trichrome staining of collagen production in wound healing in the dog (X20). (A) Control group showing the light green color of the immature collagen. B) PRP-treated group showing intense green color indicating highly mature collagen fibers. (C) PRF-treated group showing the moderate green color of collagen.

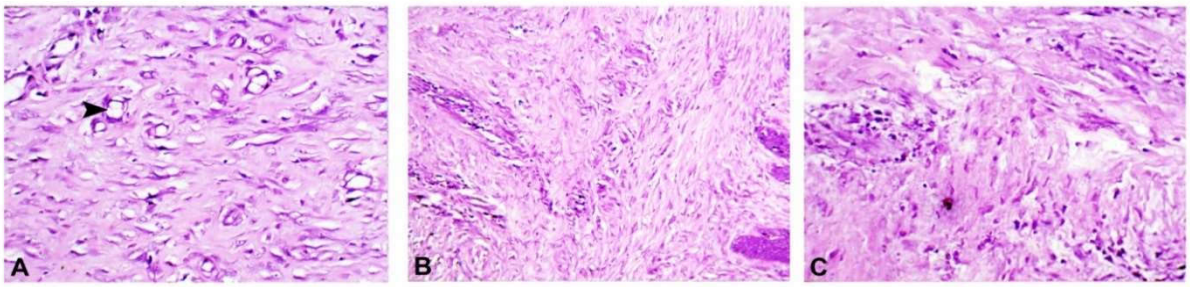

Figure 5. Neovascularization and inflammatory cellular infiltration in the dermis of skin wound healing in dog on day 21 after-surgery (H&E stain). (A) Control group showing a high number of new vessels (arrowhead) and a small number of inflammatory cells (X40). (B) PRP-treated group showing a small number of new vessels and a moderate number of inflammatory cells (X20). (C) PRF-treated group showing a small number of new vessels and a moderate number of inflammatory cells.

Table (3) indicated there was non-significant effect of wound treatment methods on body weight changes with less weight loss in treated groups than control groups. The cost of treatment ranged from LE 21 in the control group to LE 51 in PRP treated groups. Regarding the correlation between body weight changes and treatment cost and immunity represent in TGF, IL10 and WHR % results in Table (4) showed a non-significant negative low correlation

between loss of weight and WHR% and showed a significantly high positive correlation between treatment cost with IL10 (0.79*) and WHR%(0.994**). There was a particularly high positive correlation between IL10 and WHR % and a non-significant high correlation between TGF and WHR %. The results in table 5 revealed that the logarithmic function was significant (P<0.05), adjusted R^{-2} was 0.99. The average elasticity of treatment cost was about (+0.79), and the average elasticity of weight loss was about (-0.05).The results in table 6 revealed that the logarithmic function was significant (P<0.05) adjusted R^{-2} was 0.49 in TGF and 0.56 for IL10. The average elasticity of treatment cost was about (+2.19) for TGF, and the average elasticity of treatment cost was about (+3) for IL10.

Table 3 Changes in body weight (Kg/animal) and treatment cost(LE / animal) among different treated groups.

	Initial weight	Final weight	Loss of weight	Treatment cost
Control	13.67±0.33	12.67 ±0.33	1.00 ±0.01	21.00
PRP	14.00 ±0.58	13.28 ±0.53	0.72 ±0.17	51.00
PRF	13.67 ±0.88	12.85 ±0.85	0.82 ±0.09	45.00

Values are mean ± standard error (SEM). The mean differences between the values bearing different superscript letters within the same column are statistically significant (p < 0.05).

Table 4. Correlation between body weight changes, treatment cost, TGF, IL10, and WHR% of different treated groups.

	Initial weight	Final weight	Loss of weight	Treatment cost	TGF	IL10	WHR%
TGF	-0.542	-0.430	-0.480	0.614	1.000	0.323	0.654
IL10	-0.334	-0.377	0.337	0.792*	0.323	1,000	0 .740*
WHR %	-0.101	-0.076	-0.113	0.994**	0.654	0.74*	1.000

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 5 Regression between WHR% and treatment cost due to different treated methods and weight loss.

Function	Log WHR% =-0.64+0.79 log treatment cost -0.05 Log loss of weight		
T	(14.66)**	(28.41)**	(-0.87)
Sig	0.0001		
F	420		
R^{-2}	0.99		

Table 6 Regression between TGF, IL10, and treatment cost due to different treated methods.

Function	Log TGF =2.87+2.19 log treatment cost
T	(-2.48) *(2.97)*
sig	0.02
F	8.80
R-2	0.49
Function	Log IL10 =3.99+3.00 log treatment cost
T	(-2. 85) *(3.37)*
Sig	0.01
F	11.32
R-2	0.56

4. DISCUSSION

A new trend of regenerative medicine was introduced recently in second intension wound healing. The PRP and PRF represented a cornerstone in this clinical practice, where it admits many factors for the acceleration of wound healing, enhancement of wound epithelization, and neovascularization. PRP and PRF are considered excellent concentrated sources of growth factors (PDGF, TGF-β, VEGF, bFGF) and cytokines fundamental for wound healing. The wound treatment with PRP had healed rapidly and entirely within three weeks, without exuberant granulation tissue formation and minimal scarring. This is attributed to PRP used in the wound margins stimulate the production of excessive inflammatory wound mediators that provide a moist wound environment, thus increase the percentage of wound healing rate. These results coincide with previous studies (Kazemi Mehrjerdi et al. 2008).

In our study, the prepared PRP from autologous blood gave a good healing rate percent without sepsis. These results agree with that previously discussed (Farghali et al. 2019), who endorsed that the autologous PRP has many advantages of eliminating immune reaction and antimicrobial effect. Wound healing of the PRF group member showed non-significant difference from PRP-treated animals but still lower in the healing percentage rate. Our results came in agreement with previous study (Tsai et al. 2019). These reported that the PRP's liquid nature increases the power of diffusion and invasion to the tissue, so the effect is faster than PRF, which has a gel in nature.

Healing sites of the wounds take the same line with that previously mentioned (Khanzadeh Alishahi et al. 2014), who discussed that the healing wounds were almost level, and there was a relatively advanced epithelium at the wound junction of the wounds in both PRP and PRF groups as it was thicker enough in the PRP-treated group. The wound's junction was filled with dense collagen bundles as the wound edges' inner walls were united at all. Rich neovascularization was found in both groups but more abundant in PRP-treated animals.

Regarding our results, we found significant up-regulation in the expression of both IL 10 and TGF-b genes in experimental groups (PRP and PRF). This finding agrees with the study that reported that in the first six hours post wounding, there was an increase in the gene expression of mediators of inflammation such as IL-10 and growth factors including TGF-α and beta (Chamorro, Reinfeldt Engberg and Fossum 2020). During the early phase of wound healing, TGF levels were typically increased and presented at high levels at skin injury sites (James et al. 1991). This factor contributes to the recruitment of inflammatory cells, angiogenesis, collagen production, and wound remodeling (Riedel et al. 2007).

From our results, we noticed that there was a significant increase in expression of IL10 in the PRP group than the PRF group ($p < 0.05$). Also, there was significant up-regulation in the TGF-b expression in the PRF group than the PRP group ($p < 0.05$), indicating better healing in the PRP group than the PRF group with less scar formation. As it has been reported that persistent inflammatory infiltrates in chronic ulcers contribute to TGF-β levels (Finnson et al. 2013, Gilbert et al. 2016).

Also, a significantly high positive correlation between IL10 and WHR % and a non-significant high correlation between TGF and WHR % as the reduced presence of immune cells results in reduced inflammatory cytokines and growth factors such as Tgf-β1 (Sullivan et al. 1995). Moreover, increased expression of IL-10 has a role in the healing wound regeneratively with scarless healing, as it attenuates the inflammatory response (Gordon et al. 2008), acts as a regulator of the extracellular matrix (King et al. 2013), fibroblast cellular function (Leung et al. 2013), and endothelial progenitor cells (EPC) (Krishnamurthy et al. 2011).

Regarding the method of wound treatment, it had a non-significant effect on body weight changes. It showed a significantly high positive correlation between treatment cost (Cost of different methods used in wound treatment) with IL10 (0.79*) and WHR %(0.996**). Additionally, the correlation matrix showed a non-significant negative low correlation between loss of weight and WHR%. These results were in agreement with (Abegão et al. 2015), who found that non-significant changes on body weight changes and other clinical variables between control and PRP-treated groups and concerning, treatment cost as PRP in

this experiment was the highest cost and resulted in better healing rate in agreement with (Akhundov et al. 2012b) who indicated that PRP was the suitable method used for wound healing.

Regarding, the logarithmic function between WHR, loss of weight, and treatment cost (cost of different methods used in wound treatment) were significant ($P<0.001$), and about 99 % of the changes in WHR were attributed to a loss in body weight and cost of different methods used in wound treatment. The average elasticity of treatment cost was about (+0.79), meaning that increasing treatment cost by about 1 % resulted in increased WHR (0.79 %). The average elasticity of weight loss was about (-0.05%), meaning that increasing weight loss by 1% resulted in decreased WHR by (0.05%). Concerning, the logarithmic function between the cost of different methods used in wound treatment and TGF and between treatment cost and IL10 was significant ($P<0.05$), and about 49 % from the changes in TGF and 56% from changes in IL10 were attributed to differences in treatment cost due to different methods used in wound treatment. The average elasticity of treatment cost was about (+2.19), meaning that increasing treatment cost by about 1 % increased TGF (2.19 %). The average elasticity of treatment cost was about (+3%), meaning that increasing treatment cost by about 1 % resulted in increased IL10 by (3 %) this results indicated that treatment with either PRP and PRF resulted in wound healed more than normal dressing procedure as cost raised in PRP than PRF and the lowest in normal dressing this results in agreement with (Akhundov et al. 2012a) who reported that PRP could be used for the treatment of all types of wounds and was in accordance of (Gaßling et al. 2009, Bajaj et al. 2013).

5. CONCLUSION

From our clinical evaluation, molecular studies, and histopathological examination, we could conclude that the autologous PRP application has a better healing percentage rate than PRF. PRP accelerates wound healing through the enhancement of wound re-epithelization, neo-vascularization and reduce scar formation. Research on the extended implications of TGF-β signaling and IL10 in wounds studied revealed a significant increase in expression of IL10 in the PRP group than PRF group. Also, there was significant up-regulation in TGF-b expression in the PRF group than the PRP group.

ACKNOWLEDGMENT

This work was supported by the research project entitled (Assessment of expression of wound healing genes after treatment with Platelet Rich Plasma (PRP) & Synthetic oligodeoxynucleotides (CpG-ODN) using PCR-Array technique: new trend for treatment & genetic evaluation) and authors extended their appreciation to the scientific research fund (SRF) at Benha University, for their fund project no (M\5\1\5). The authors also would like to thank Prof. Dr. Shawky Moustafa for the histopathological examinations.

AUTHORS'S CONTRIBUTIONS

Conceptualization, design, supervision: Olla A. Khalifa. Methodology, investigation, resources, data curation, writing original draft preparation, review and editing : Olla A. Khalifa, Abdelhaleem H. Elkasapy, Eman A. Sallam, Adel M. Alakraa, Yasmin M. Marei and Liza S. Mohammed. All authors have read and agreed to the published version of the manuscript

6. REFERENCES

1. Abegão, K. G. B., B. N. Bracale, I. G. Delfim, E. S. d. Santos, C. B. Laposy, G. A. Nai, R. Giuffrida and Nogueira, R. M. B. (2015) Effects of heterologous platelet-rich plasma gel on standardized dermal wound healing in rabbits. *Acta cirúrgica brasileira,* 30, 209-215.
2. Akhundov, K., G. Pietramaggiori, L. Waselle, S. Darwiche, S. Guerid, C. Scaletta, N. Hirt-Burri, L. A. Applegate and W. V. Raffoul (2012a) Development of a cost-effective method for platelet-rich plasma (PRP) preparation for topical wound healing. *Ann Burns Fire Disasters,* 25, 207-13.
3. Akhundov, K., G. Pietramaggiori, S. D. Waselle, S. Guerid, C. Scaletta, N. Hirt-Burri, L. Applegate and W. Raffoul (2012b) Development of a cost-effective method for platelet-rich plasma (PRP) preparation for topical wound healing. *Annals of burns and fire disasters,* 31; 25(4): 207–213.
4. An, L., G. Q. Dong, Q. Gao, Y. Zhang, L. W. Hu, J. H. Li and Y. Liu (2010) Effects of UVA on TNF-α, IL-1β, and IL-10 expression levels in human keratinocytes and intervention studies with an antioxidant and a JNK inhibitor. *Photodermatology, photoimmunology & photomedicine,* 26, 28-35.
5. Bajaj, P., A. Pradeep, E. Agarwal, N. S. Rao, S. B. Naik, N. Priyanka and N. Kalra (2013) Comparative evaluation of autologous platelet-rich fibrin and platelet-rich plasma in the treatment of mandibular degree II furcation defects: a randomized controlled clinical trial. *Journal of periodontal research,* 48, 573-581.
6. Bancroft, J. D. and M. Gamble. 2008. *Theory and practice of histological techniques*. 6th edition Elsevier health sciences.
7. Bennett, R. & J. IJpelaar (2005) Updated Estimates of the Costs Associated with Thirty Four Endemic Livestock Diseases in Great Britain: A Note. *Journal of Agricultural Economics,* 56, 135-144.
8. Bielecki, T., T. Gazdzik, J. Arendt, T. Szczepanski, W. Krol and T. Wielkoszynski (2007) Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: an in vitro study. *The Journal of bone and joint surgery. British volume,* 89, 417-420.
9. Bielefeld, K. A., S. Amini-Nik and B. A. Alman (2013) Cutaneous wound healing: recruiting developmental pathways for regeneration. *Cellular and Molecular Life Sciences,* 70, 2059-2081.
10. Brancato, S. K. and J. E. Albina (2011) Wound macrophages as key regulators of repair: origin, phenotype, and function. *The American journal of pathology,* 178, 19-25.
11. Brinkhof, B., B. Spee, J. Rothuizen and L. C. Penning (2006) Development and evaluation of canine reference genes for accurate quantification of gene expression. *Analytical biochemistry,* 356, 36-43.
12. Chamorro, C. I., G. Reinfeldt Engberg and M. Fossum (2020) Molecular and histological studies of bladder wound healing in a rodent model. *Wound Repair and Regeneration,* 28, 293-306.
13. Dohan, D. M., J. Choukroun, A. Diss, S. L. Dohan, A. J. Dohan, J. Mouhyi and B. Gogly (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology,* 101, e37-e44.
14. Dohan Ehrenfest, D. M., M. Del Corso, A. Diss, J. Mouhyi and J. B. Charrier (2010) Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. *Journal of periodontology,* 81, 546-555.
15. Eldebany, N., M. Abd Elkodous, H. Tohamy, R. Abdelwahed, M. El-Kammar, H. Abou-Ahmed and H. Elkhenany (2020) Gelatin Loaded Titanium Dioxide and Silver Oxide Nanoparticles: Implication for Skin Tissue Regeneration. *Biological Trace Element Research*,199:3688–3699.
16. Esat, D. M., S. Temel, H. Ozer, U. M. Kemal, F. Kaya, F. Aslan, P. Celepli, M. Senes, K. Dogan and S. Kuru (2016)

Comparison of the Effects of Plateletrich Plasma Prepared in Various Forms on the Healing of Dermal Wounds in Rats. *Wounds: a compendium of clinical research and practice,* 28(3): 99-108.

17. Farghali, H. A., N. A. AbdElKader, H. O. AbuBakr, S. H. Aljuaydi, M. S. Khattab, R. Elhelw and M. Elhariri (2019) Antimicrobial action of autologous platelet-rich plasma on MRSA-infected skin wounds in dogs. *Scientific reports,* 9, 1-15.
18. Farghali, H. A., N. A. AbdElKader, M. S. Khattab and H. O. AbuBakr (2017) Evaluation of subcutaneous infiltration of autologous platelet-rich plasma on skin-wound healing in dogs. *Bioscience reports,* 37(2):BSR20160503. Published 2017 Apr 10. doi:10.1042/BSR20160503
19. Finnson, K. W., S. McLean, G. M. Di Guglielmo & A. Philip (2013) Dynamics of transforming growth factor beta signaling in wound healing and scarring. *Advances in wound care,* 2, 195-214.
20. Fortunato, S. J., R. Menon and S. J. Lombardi (1998) The effect of transforming growth factor and interleukin-10 on interleukin-8 release by human amniochorion may regulate histologic chorioamnionitis. *American journal of obstetrics and gynecology,* 179, 794-799.
21. Gaßling, V. L., Y. Açil, I. N. Springer, N. Hubert and J. Wiltfang (2009) Platelet-rich plasma and platelet-rich fibrin in human cell culture. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology,* 108, 48-55.
22. Gilbert, R. W., M. K. Vickaryous and A. M. Viloria-Petit (2016) Signalling by transforming growth factor beta isoforms in wound healing and tissue regeneration. *Journal of developmental biology,* 22;4(2):21. doi: 10.3390/jdb4020021.
23. Gordon, A., E. D. Kozin, S. G. Keswani, S. S. Vaikunth, A. B. Katz, P. W. Zoltick, M. Favata, A. P. Radu, L. J. Soslowsky and M. Herlyn (2008) Permissive environment in postnatal wounds induced by adenoviral-mediated overexpression of the anti-inflammatory cytokine interleukin-10 prevents scar formation. *Wound Repair and Regeneration,* 16, 70-79.
24. Han, G., F. Li, T. P. Singh, P. Wolf and X.-J. Wang (2012) The pro-inflammatory role of TGFβ1: a paradox? *International journal of biological sciences,* 8(2):228-35
25. James, L. C., A. M. Moore, L. A. Wheeler, G. M. Murphy, P. M. Dowd and M. W. Greaves (1991) Transforming growth factor α: In vivo release by normal human skin following UV irradiation and abrasion. *Skin Pharmacology and Physiology,* 4, 61-64.
26. Kallis, P. J. & A. J. Friedman (2018) Collagen Powder in Wound Healing. *Journal of drugs in dermatology: JDD,* 17, 403-408.
27. Kazemi Mehrjerdi, H., K. Sardari, M. R. Emami, A. R. Movassaghi, A. Afkhami Goli, A. Lotfi and S. Malekzadeh (2008) Efficacy of autologous platelet-rich plasma (prp) activated by thromboplastin-D on the repair and regeneration of wounds in dogs. *Iranian Journal of Veterinary Surgery,* 3, 19-30.
28. Khanzadeh Alishahi, M., D. Kazemi, D. Mohajeri, H. Mofidpoor, A. Afkhami Goli and M. A. Khanzadeh Alishahi (2014) Histopathological evaluation of the effect of platelet-rich fibrin on canine cutaneous incisional wound healing. *Iranian Journal of Veterinary Science and Technology,* 5, 19-32.
29. Kimura, A., H. Ogata, M. Yazawa, N. Watanabe, T. Mori and T. Nakajima (2005) The effects of platelet-rich plasma on cutaneous incisional wound healing in rats. *Journal of Dermatological Science,* 40, 205-208.
30. King, A., S. Balaji, E. Marsh, L. D. Le, A. F. Shaaban, T. M. Crombleholme and S. G. Keswani (2013) Interleukin-10 regulates the fetal hyaluronan-rich extracellular matrix via a STAT3-dependent mechanism. *Journal of Surgical Research,* 184, 671-677.
31. Krishnamurthy, P., M. Thal, S. Verma, E. Hoxha, E. Lambers, V. Ramirez, G. Qin, D. Losordo and R. Kishore (2011) Interleukin-10 deficiency impairs bone marrow–derived endothelial progenitor cell survival and function in ischemic myocardium. *Circulation research,* 109, 1280-1289.
32. Kulkarni, A. B., C.-G. Huh, D. Becker, A. Geiser, M. Lyght, K. C. Flanders, A. B. Roberts, M. B. Sporn, J. M. Ward and S. Karlsson (1993) Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proceedings of the National Academy of Sciences,* 90, 770-774.
33. Leung, A., S. Balaji, L. Le, E. Marsh, Y. Dhamija, I. Sharma-Crawford, M. Habli, T. Crombleholme and S. Keswani (2013) Interleukin-10 and hyaluronan are essential to the fetal fibroblast functional phenotype. *Journal of Surgical Research,* 179, 257-258.
34. Livak, K.J. and T.D. Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. Methods, 25: 402-408.
35. Mishra, A., J. Woodall Jr and A. Vieira (2009) Treatment of tendon and muscle using platelet-rich plasma. *Clinics in sports medicine,* 28, 113-125.
36. Moore, K. W., R. de Waal Malefyt, R. L. Coffman and A. O'Garra (2001) Interleukin-10 and the interleukin-10 receptor. *Annual review of immunology,* 19, 683-765.
37. Nikolidakis, D. and J. A. Jansen (2008) The biology of platelet-rich plasma and its application in oral surgery: literature review. *Tissue Engineering Part B: Reviews,* 14, 249-258.
38. Riedel, K., F. Riedel, U. R. Goessler, G. Germann and M. Sauerbier (2007) TGF-β antisense therapy increases angiogenic potential in human keratinocytes in vitro∗. *Archives of medical research,* 38, 45-51.
39. Sato, T., M. Yamamoto, T. Shimosato & D. M. Klinman (2010) Accelerated wound healing mediated by activation of Toll-like receptor 9. *Wound Repair and Regeneration,* 18, 586-593.
40. Shull, M. M., I. Ormsby, A. B. Kier, S. Pawlowski, R. J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel and D. Calvin (1992) Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature,* 359, 693-699.
41. Singer, A. J. and R. A. Clark (1999) Cutaneous wound healing. *New England journal of medicine,* 341, 738-746.
42. Spee, B., L. C. Penning, T. S. van den Ingh, B. Arends, J. IJzer, F. J. van Sluijs and J. Rothuizen (2005) Regenerative and fibrotic pathways in canine hepatic portosystemic shunt and portal vein hypoplasia, new models for clinical hepatocyte growth factor treatment. *Comparative Hepatology,* 4, 7. doi: 10.1186/1476-5926-4-7
43. SPSS, I. (2007) SPSS version 16.0. *Chicago, IL: SPSS Incorporated*.
44. Sullivan, K. M., H. P. Lorenz, M. Meuli, R. Y. Lin and N. S. Adzick (1995) A model of scarless human fetal wound repair is deficient in transforming growth factor beta. *Journal of pediatric surgery,* 30, 198-203.
45. Tsai, H.-C., G. R.-L. Chang, H.-C. Fan, H. Ou-Yang, L.-C. Huang, S.-C. Wu and C.-M. Chen (2019) A mini-pig model for evaluating the efficacy of autologous platelet patches on induced acute full thickness wound healing. *BMC veterinary research,* 7;15(1):191.
46. Veenhof, E. Z., V. P. Rutten, R. van Noort, E. F. Knol and T. Willemse (2010) Evaluation of T-cell activation in the duodenum of dogs with cutaneous food hypersensitivity. *American journal of veterinary research,* 71, 441-446.
47. Villela, D. L. and V. L. C. Santos (2010) Evidence on the use of platelet-rich plasma for diabetic ulcer: a systematic review. *Growth factors,* 28, 111-116.
48. Waelbers, T., P. Vermoere and I. Polis (2009) Total intravenous anesthesia in dogs. *Vlaams Diergeneeskundig Tijdschrift,* 78, 160-169.
49. Wang, X.-J., G. Han, P. Owens, Y. Siddiqui and A. G. Li. 2006. Role of TGFβ-mediated inflammation in cutaneous wound healing. In *Journal of Investigative Dermatology Symposium Proceedings*, 112-117.
50. Werner, S. and R. Grose (2003) Regulation of wound healing by growth factors and cytokines. *Physiological reviews* ;83(3):835-70..