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IMMUNO-HEMATOLOGICAL RESPONSE TO RADIAL NERVE INJURY AND HUMAN UMBILICAL CORD-MESENCHYMAL STEM CELLS (HUC-MSCS) THERAPY IN DOGS, IRAQ

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ABSTRACT : The current study was aimed to evaluate therapeutic efficacy of human umbilical cord-mesenchymal stem cells (HUC-MSCs) to repair of radial nerve injury through estimation of clinical status, serum biomarkers and hematology. A total 18 stray, male dogs of 12-18 months of age and 8-10Kg of weight were selected, examined clinically, subjected to a preparation period, and divided into 3 equal groups; 1stG (experimental group) in which radial nerve of study animals was cut surgically and treated with HUC-MSCs; 2ndG (positive control group) in which, radial nerve of study animals was cut, but not treated with HUC-MSCs, and the 3rdG (negative Control group) in which radial nerve neither cut nor treated with HUC-MSCs. During 16 weeks, all study dogs were feed and let drink from the same source and received a high management care. In 1stG, significant motive dysfunction with apparent decline in appetite, changes in sleeping habits, slight increase in temperature, shallow and rapid breathing, and slight tachycardia were showed in 1st week. During 2nd to 5th weeks, there was apparent improvement in general and physical activity with the ability of 1stG animals to extend their forelimb and standup normally, but with lameness. At 16th week, clinical status was normal with absence of forelimb muscle atrophy, lameness, and motive dysfunction. Concerning to immune-biomarkers, significant increases (P<0.05) were reported in levels of IgG and TNF-á and significant reduction (P<0.05) in IL-10 were detected at 4th and 6th, 2nd and 4th and 2nd, 4th and 6th weeks respectively. Regarding to hematology, there were significant increases in total WBCs, lymphocytes and monocytes but not in neutrophils (P>0.05) were reported at 4th and 6th weeks of study. In conclusion, positive outcomes achieved in this study suggesting effective role of HUC-MSCs in repairing of radial NI and offered a great promise for some disease treatment.

Key words : Immune biomarkers, radial nerve, stem cells, dogs, hematology, Iraq.

INTRODUCTION

In small animal practice, traumatic nerve injury (NI) is quite common as it observed in animal at any age (Antolitou *et al*, 2012). There two mode for classification are available for NI. In first mode, NI can be divided into three main forms; neuropraxia, axonotmesis, and neurotmesis which differ in their severity and outcomes (Seddon, 1943). In neuropraxia, there is a minor contusion or compression for the nerve with a temporary interruption in transmission of electrical impulses that will typically resolve by 3 to 6 weeks (Bumbasirevic *et al*, 2016). Axonotmesis represents a more severe form of nerve injury with damage to the axons accompanying distal Wallerian degeneration, but maintaining perseveration of Schwann cells and an intact endoneurial structure (Tezcan, 2017). Neurotmesis is the most severe form of NI which characterized by the complete anatomical disruption to nerve continuity with proliferation of fibrous tissue, Schwann cells and axonal growth resulting in losses of nerve architecture and function. In last form, there is no possibility of spontaneous nerve recovery, so that, surgical interpretation is necessary (Olaifa, 2018). In other mode, there were five degrees of NI which classified based on severity of NI as the first-degree represents more simple case while the fifth represent the more severe one (Sunderland, 1968). After NI, there many limitations for functional recovery such as motor end-plate degeneration, neuromuscular atrophy, adhesion of regenerative nerve to peripheral tissues, slow neural regeneration velocity and complex pathological processes; so clinical therapeutic effects are not satisfactory (Caldwell *et al*, 2013; Korompilias *et al*, 2013; Niver and Ilyas, 2013). At present, for treatment of peripheral NI, the most common method with optimal therapeutic effect is end-to-end anastomosis or nerve auto-grafting with variable limitation for each one (Li *et al*, 2013). Human umbilical cord-mesenchymal stem cells (HUC-MSCs) are one of the most common seed cells used in nerve tissue

engineering which revealed strong self-renewal and multi-differentiation potentials and can be induced to differentiate into nerve cells *in vitro* (Ning *et al*, 2012).

During injury and therapeutic processes, resident immune cells are activated and blood-borne cells are recruited to the site of injury. As a result, the primary and secondary lymphoid organs received an extensive sympathetic/noradrenergic innervation which leads to dysfunction of homeostatic mechanisms and hematological changes in acute and chronic phases (Madden *et al*, 2000; Furlan *et al*, 2006). Therefore, the current study was performed to evaluate therapeutic efficiency of HUC-MSCs in repair of radial NI through estimation of clinical status, some immune biomarkers, and describe the hematological abnormalities during radial NI and HUC-MSCs therapy.

MATERIALS AND METHODS

Ethical approval

This study was licensed and performed under the regulation of Department of Microbiology and Department of Surgery and obstetrics in the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. HUC collection was approved by the Scientific and Ethical Committee of Baghdad University.

Study animals

A total of 18 stray, male dogs of 12-18 months of age and 8-10 Kg of weight were selected for this study. Prior to the experimental study, all study dogs were examined clinically to detect any diseases, treated against external and internal parasites and subjected to a preparation period for one month with providing good environmental and management hygienic conditions.

Stem cells preparation

Following a sterile environment, HUC was collected from one healthy woman immediately after given birth and transported to the laboratory as soon as possible. According to method described previously (Xue *et al*, 2011). Briefly, UC was cut into approximately 1.5cm pieces, gelatinous tissue surrounding the vessels was excised and minced into 1mm3 which plated in closed cell culture flasks. After 20 days, the adherent spindle-shaped cells were collected and re-cultured for some passages to be used for treatment of radial nerve injury.

Study design

Post preparation period finished, the study dogs were divided into 3 equal groups (6 dogs for each one) in solitary cages as following:

1. First group (1stG): In which, radial nerve was cut, and treated with HUC-MSCs (Experimental group).
2. Second group (2ndG): In which, radial nerve was cut, but not treated (Positive Control group).
3. Third group (3rdG): In which, radial nerve neither cut, nor treated with HUC-MSCs (Negative Control group).

Experimental study period was continued for 16 weeks (October, 2018 to May, 2019); during which, all dogs were feed and let drink from the same source, and received a high management care.

Radial NI and cell therapy

Following high constricted hygienic conditions, the dogs of experimental and positive groups were fully anesthetized and subjected surgically for cutting of radial nerve to make a gap of 1cm between the ends of the nerve (Fig. 1A-D). In experimental dogs, the gaps between the ends of radial nerve was tabularized by conduit and filled by HUC-MSCs (Fig. 1E, F); whereas the dogs of positive control were left without HUC-MSCs therapy. After that, surgical wounds were sutured, sterilized, and the dogs were subjected for post operative care (Fig. 1).

Clinical examination

To detect the HUC-MSCs therapeutic effects, clinical examination before and after therapy was performed, and this included general and physical evaluation of study dogs and their activities and motive function.

Hematology

From each study dog of three groups, 2.5ml of venous blood was collected into EDTA-glass tubes throughout the period of experimental study at two weeks intervals. Blood parameters including total WBCs count, lymphocytes, neutrophils, and monocytes were measured as soon as possible using the automated Mythic18 Vet blood analyzer (Orphee´, Switzerland). Then, tubes of anticoagulant blood were centrifuged (3000rpm/5 min), and the serum samples were kept frozen in numbered eppendorf tubes until be used for immunology.

Immunology

In this study, canine immunoglobulin (IgG), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) were measured in serum samples using of specific Sandwich ELISA kits (Sunlong Biotech, China). According to manufacturer's instruction, the Standards were diluted serially and Dilution Buffer was prepared. At terminal stage of the assay, absorbance optical density (OD) was read at 450nm using ELISA Microplate Reader (Bio Tek, USA). Finally, concentrations of immune biomarkers (IgG, IL-10 and TNF-α) were calculated by the log scale (x-axis and y-axis) and multiplying of dilution

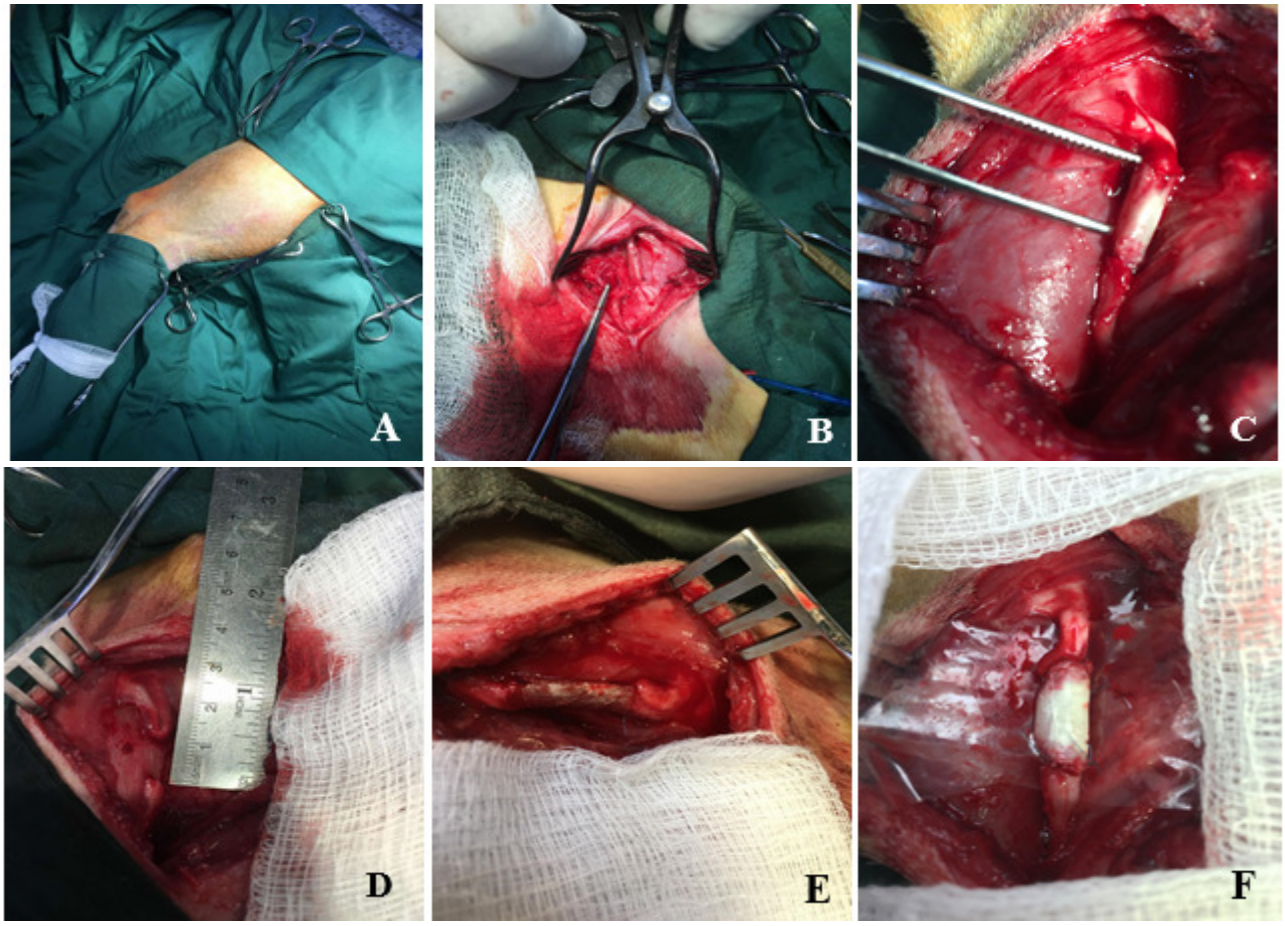

Fig. 1 : Surgical procedure for radial NI and HUC-MSCs therapy.

buffer.

Statistical analysis

All study data were tabled and analyzed using the Microsoft Office Excel (version 2007) and IBM-SPSS programs (version 16). One-Way ANOVA was used to statistical evaluation of hematological and immunological values between the three study groups (Kassambara, 2017). Significant differences were set at a probability of $P<0.05$.

RESULTS

Among study groups, findings of clinical examination, hematology, and immunology were revealed on significant variation ($P<0.05$) in their values.

Clinical evaluation

Before surgery, all study dogs were evaluated to having normal general and physical activities. At the first week of experimental study, the dogs of 1stG were showed a significant motive dysfunction with apparent decline in appetite, changes in sleeping habits, slight increase in temperature, shallow and rapid breathing, and slight tachycardia. During 2nd week up to 5th week, there was apparent improvement in general and physical activity of 1stG dogs, with the ability of their animals to extend their forelimb and standup normally but with lameness throughout moving (Fig. 2A). At the final week of study, 16th week, general and physical activities were normal for the dogs of 1stG with absence of forelimb muscle atrophy and the signs of lameness and ability of these animals to extend their forelimbs normally without any motive dysfunction (Fig. 2B) (Fig. 2).

Immunology

Concerning to levels of IgG in dogs of 1st G, the highest significant increases ($P<0.05$) were reported at the 4th and 6th weeks rather than other weeks. However, significant decline ($P<0.05$) was appeared at the 8th and 16th week. Among groups, significant increases ($P<0.05$) were detected in the 2nd G which followed by the 1st G in comparison to the 3rd G (Table 1, Fig. 3-A).

For IL-10, there significant decreases ($P<0.05$) were showed in animals of 1s G at the 2nd and 4th weeks, which followed by slight significant increases ($P<0.05$) at the 6th, 8th and 16th weeks. Among study groups, significant decreases ($P<0.05$) were initiated at 4th week in 1st G and 2nd G in comparison to 3rd G. However, the levels of IL-10 were showed significant elevation ($P<0.05$) at other

Fig. 2 : Clinical status for one of study dog subjected to radial NI and HUC-MSCs therapy.

Table 1 : Total results of IgG among groups of dogs and weeks of the study.

Week	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	2.92 ± 0.06 ^{Ac} (2.80-3.25)	2.77 ± 0.08 ^{Ac} (2.64-3.19)	2.79 ± 0.14 ^{Aa} (2.61-3.19)
2	3.9 ± 0.2 ^{Bb} (3.17-4.58)	5.24 ± 0.14 ^{Ab} (4.75-5.98)	2.83 ± 0.14 ^{Ca} (2.49-3.28)
4	4.68 ± 0.26 ^{Ba} (4.39-5.46)	5.61 ± 0.2 ^{Ab} (4.81-6.27)	2.74 ± 0.13 ^{Ca} (2.64-3.50)
6	4.36 ± 0.19 ^{Ba} (4.21-5)	5.88 ± 0.21 ^{Aa} (5.07-6.89)	2.82 ± 0.11 ^{Ca} (2.54-3.29)
8	4.09 ± 0.11 ^{Bb} (4.02-4.64)	6.03 ± 0.28 ^{Aa} (5.46-7.11)	2.77 ± 0.15 ^{Ca} (2.60-3.54)
16	3.83 ± 0.14 ^{Bb} (3.72-4.24)	6.32 ± 0.26 ^{Aa} (5.84-7.36)	2.84 ± 0.12 ^{Ca} (2.57-3.40)

Variation in large horizontal and small vertical letters refer to significance (P<0.05)

Table 2 : Total results of IL-10 among groups of dogs and weeks of the study.

Week	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	70.21 ± 1.94 ^{Aa} (67.61-75.46)	70.98 ± 2.72 ^{Aa} (65.18-73.95)	69.34 ± 2.17 ^{Aa} (59.19-71.49)
2	59.66 ± 2.08 ^{Ac} (55.18-64.21)	58.77 ± 1.24 ^{Aa} (56.31-62.17)	69.75 ± 2.91 ^{Aa} (57.25-72.30)
4	56.5 ± 2.21 ^{Cc} (54.25-61.79)	53.12 ± 1.58 ^{Bb} (51.29-58.13)	70.03 ± 2.04 ^{Aa} (57.89-70.68)
6	59.13 ± 1.93 ^{Bb} (57.48-64.32)	52.67 ± 1.62 ^{Bb} (50.68-56.02)	69.84 ± 59.03 ^{Aa} (59.03-71.65)
8	61.05 ± 2.11 ^{Bb} (58.31-65.88)	49.38 ± 1.37 ^{Cc} (46.79-55.11)	69.91 ± 1.53 ^{Aa} (60.11-72.14)
16	63.2 ± 2.73 ^{Bb} (60.49-69.87)	46.78 ± 2.08 ^{Cd} (41.54-50.79)	68.76 ± 2.02 ^{Aa} (58.38-71.97)

Variation in large horizontal and small vertical letters refer to significance (P<0.05)

weeks (6th, 8th and 16th) in 1st G in contrast to 2nd G which continued in significant decline at the same weeks (Table 2, Fig. 3-B).

Regarding to TNF-á, the findings of 1st G were reported significant elevation (P<0.05) in their concentrations particularly in 2nd, 4th and 6th weeks. Among groups, significant increases (P<0.05) in TNF- á concentrations of 1st G were detected at the 4th and 6th weeks in comparison to 3rd G (Table 3, Fig. 3-C).

Values of hematology measured in current study were revealed on significant differences (P<0.05) in their levels. For total WBCs, there was significant elevation (P<0.05) at 4th and 6th weeks of 1st G, whilst among groups, significant decreases (P<0.05) in values of 1st G were detected at 4th, 6th, 8th, and 16th weeks in comparison to 3rd G; and significant increases (P<0.05) in relation to 2nd G (Table 4, Fig. 4-A).

Table 3 : Total results of TNF-á among groups of dogs and weeks of the study.

Week	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	55.11 ±1.52^{Ac} (50.43-59.28)	54.59 ± 1.42 ^{Ac} (49.23-58.64)	55.41 ± 1.17^{Aa} (49.87-58.43)
2	65.18 ± 1.06 ^{Aa} (63.83-67.29)	64.73 ± 1.72^{Ab} (58.67-66.85)	54.96 ± 0.55^{Aa} (50.09-57.24)
4	69.26 ± 1.23^{Aa} (66.05-74.81)	67.01 ± 2.03^{Ab} (63.54-68.12)	53.45 ± 2.4 ^{Ba} (48.92-59.03)
6	67.55 ± 1.45 ^{Aa} (62.27-71.16)	69.53 ± 1.75^{Aa} (64.45-70.09)	54.15 ± 1.26^{Ba} (49.11-57.91)
8	63.17 ± 1.12 ^{Bb} (58.31-69.46)	70.39 ± 1.46 ^{Aa} (65.34-72.88)	55.53 ± 1.39^{Ba} (49.67-58.88)
16	59.68 ± 2.31^{Bb} (53.14-66.28)	69.45 ± 2.11^{Aa} (64.14-73.04)	55.21 ± 2.04 ^{Ba} (48.71-58.63)

Variation in large horizontal and small vertical letters refer to significance (P<0.05).

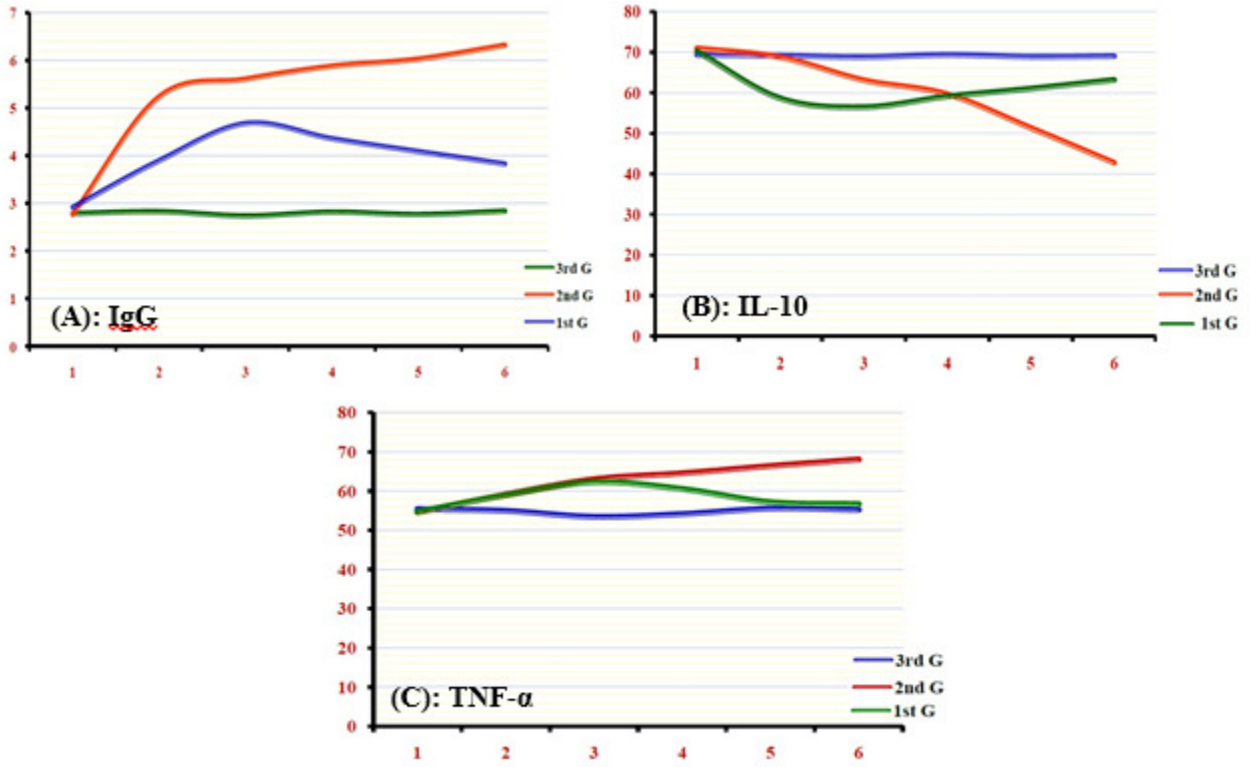

Fig. 3 : Levels of immunological biomarkers among dogs of study groups and weeks.

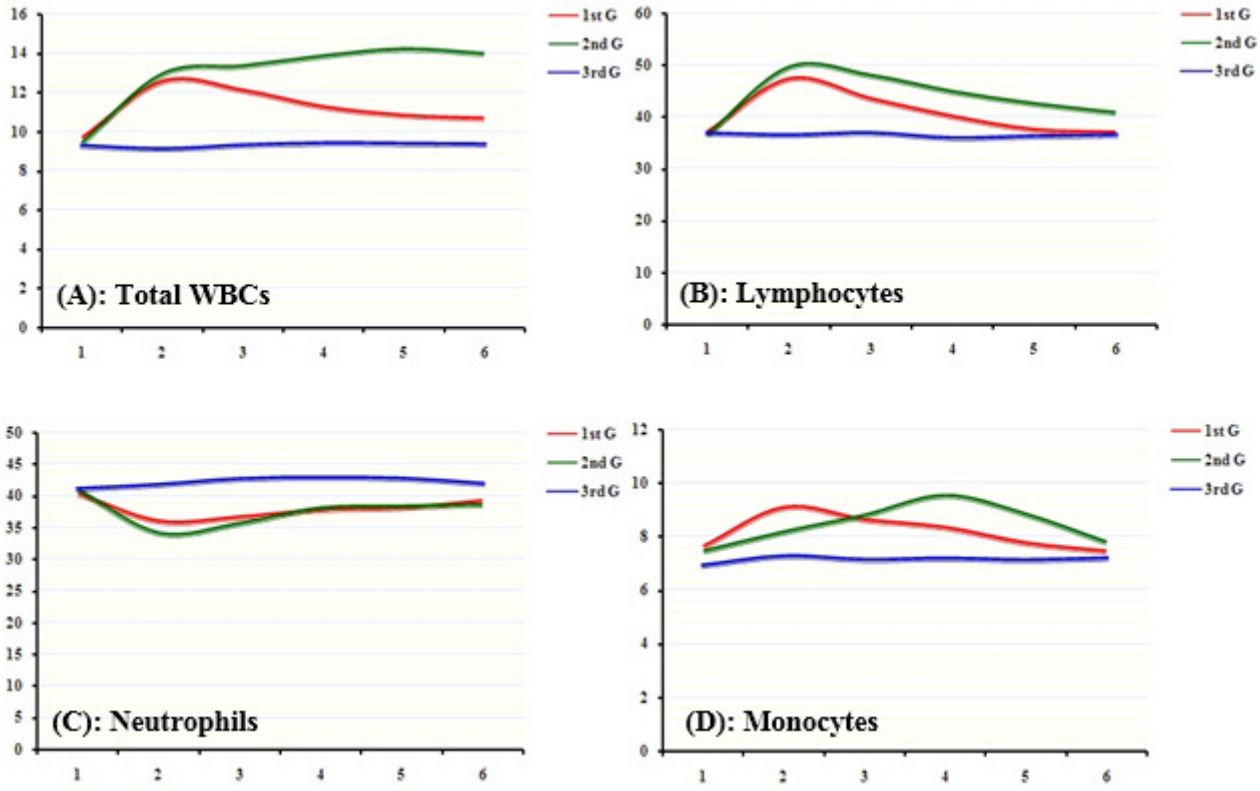

Fig. 4 : Levels of hematological parameters among dogs of study groups and weeks.

In lymphocytes, significant increases (P<0.05) were showed in 1st G at 2nd and 4th weeks rather than other weeks; whilst among groups, significant increases (P<0.05) in values of 1st G were reported at 2nd, 4th, and 6th weeks in comparison to 3rd G (Table 5, Fig. 4-B).

No significant differences (P>0.05) in levels of neutrophils were seen among weeks of 1st G, or in comparison to other groups, 2nd G and 3rd G (Table 6, Fig. 4-C). Concerning to monocytes, a slight significant elevation (P<0.05) was detected at 2nd and 4th weeks of

Table 4 : Total results of total WBCs among groups of dogs and weeks of the study.

Week (Unite×10^3/µl)	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	9.69±0.18^{Ad} (7.98-10.82)	9.41 ± 0.17^{Ad} (7.72-10.99)	9.28 ± 0.21^{Aa} (7.59-11.01)
2	12.54 ± 0.23^{Aa} (9.73-14.26)	12.94 ± 0.31^{Ac} (11.35-14.16)	9.11 ± 0.17^{Ba} (7.41-11.99)
4	12.08 ± 0.18 ^{Ba} (10.47-14.51)	13.35 ± 0.27^{Ab} (10.94-14.46)	9.31 ± 0.19^{Ca} (7.66-12.13)
6	11.25 ± 0.14^{Bb} (9.88-13.79)	13.86 ± 0.27^{Aa} (11.28-14.51)	9. 41 ± 0.15^{Ca} (7.49-12.08)
8	10.82 ± 0.16^{Bb} (7.65-13.04)	14.22 ± 0.19^{Aa} (11.37-14.72)	9.39 ± 0.18^{Ca} (7.51-11.97)
16	10.67 ± 0.15^{Bc} (7.78-13.61)	13.98 ± 0.22^{Aa} (10.40-15.03)	9.36 ± 0.16 ^{Ca} (7.63-12.01)

Variation in large horizontal and small vertical letters refer to significance (P<0.05).

Table 5 : Total results of lymphocytes among groups of dogs and weeks of the study.

Week (Unite %)	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	36.91 ± 1.82^{Ab} (32.41-39.51)	36.56 ± 1.48^{Ab} (31.98-38.41)	36.87 ± 2.02^{Aa} (32.72-39.63)
2	47.24 ± 1.97^{Aa} (40.39-51.45)	49.49 ± 2.06^{Aa} (42.77-52.29)	36.44 ± 1.49^{Ba} (32.21-39.54)
4	43.38 ± 2.03^{Aa} (38.29-47.16)	47.92 ± 1.25^{Aa} (40.83-51.74)	36.92 ± 1.82^{Ba} (33.15-39.30)
6	39.95 ± 2.23^{Ab} (36.02-42.56)	44.81 ± 1.37^{Aa} (39.25-47.99)	35.81 ± 1.27^{Ba} (32.62-38.75)
8	37.37 ± 1.91^{Bb} (35.03-39.92)	42.51 ± 1.19^{Ab} (38.80-44.96)	36.22 ± 1.35^{Ba} (32.81-39.10)
16	36.83 ± 1.74^{Ab} (34.78-38.51)	40.79 ± 1.33^{Ab} (37.12-42.16)	36.48 ± 1.31^{Aa} (32.71-38.54)

Variation in large horizontal and small vertical letters refer to significance (P<0.05).

Table 6 : Total results of neutrophils among groups of dogs and weeks of the study.

Week (Unite %)	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	40.27 ± 2.30^{Aa} (37.76-43.31)	40.81 ± 2.51^{Aa} (37.19-42.51)	41.09 ± 1.99^{Aa} (37.28-43.19)
2	35.91 ± 1.86^{Aa} (33.82-37.25)	34.06 ± 1.97^{Ab} (33.66-37.61)	41.75 ± 2.22^{Aa} (37.65-44.13)
4	36.55 ± 1.91^{Aa} (34.11-38.57)	36.87 ± 1.81^{Ab} (34.74-39.01)	42.67 ± 1.84^{Aa} (38.06-43.92)
6	37.72 ± 2.06^{Aa} (34.78-39.21)	37.99 ± 1.63^{Aa} (35.26-39.53)	42.88 ± 2.06^{Aa} (39.61-43.75)
8	38.03 ± 1.74^{Aa} (34.88-39.92)	38.26 ± 1.72^{Aa} (35.22-39.94)	42.73 ± 1.73^{Aa} (38.14-44.08)
16	39.14 ± 2.13^{Aa} (36.72-41.54)	38.51 ± 1.69^{Aa} (36.78-41.26)	41.92 ± 1.81^{Aa} (38.03-44.21)

Variation in large horizontal and small vertical letters refer to significance (P<0.05).

Table 7 : Total results of monocytes among groups of dogs and weeks of the study.

Week (Unite %)	1st G M ± SE (R)	2nd G M ± SE (R)	3rd G M ± SE (R)
0	7.61 ± 0.37^{Ac} (6.22-8.91)	7. 42 ± 0.31^{Ad} (5.57-9.22)	6.89 ± 0.29^{Aa} (5.65-9.34)
2	9.07 ± 0.33^{Aa} (6.19-9.74)	8.15 ± 0.29^{Bc} (6.42-10.23)	7.25 ± 0.23^{Ca} (5.42-8.87)
4	8.61 ± 0.29^{Aa} (7.18-9.41)	8.78 ± 0.23^{Ab} (8.02-11.18)	7.11 ± 0.24^{Ba} (5.39-9.02)
6	8.32 ± 0.31^{Bb} (7.02-9.11)	9.53 ± 0.27^{Aa} (7.84-11.25)	7.16 ± 0.26^{Ca} (5.45-8.87)
8	7.74 ± 0.32^{Bc} (5.84-8.77)	8.82 ± 0.34^{Ab} (7.15-11.38)	7.10 ± 0.23^{Ca} (5.78-9.00)
16	7.43 ± 0.30^{Ac} (5.62-8.54)	7.76 ± 0.27^{Ad} (6.83-10.15)	7.17 ± 0.25^{Ba} (5.64-8.97)

Variation in large horizontal and small vertical letters refer to significance (P<0.05)

1st G in comparison to other weeks, and that same weeks comparing to other groups (Table 7, Fig. 4-D).

DISCUSSION

Peripheral NI generally as radial nerve, refers to a structural and functional impairment, even excessive discontinuance caused by drag, pressurization or transaction leading to a series of dysfunctions in affected region (Li *et al*, 2013). Treatment of NI and regeneration remains among the greatest challenges in tissue engineering and regenerative medicine because fail is seen even when the best microsurgical technique is applied (De Carvalho *et al*, 2019). However, there many factors can determine the time which elapses between suture and recovery such as scar delay or latent period, and rate of progress down the nerve of the processes of maturation or functional completion of the axons (Wu *et al*, 2013). In humans and animals, many techniques are used mainly in the management of certain neuropathies associated with locomotors dysfunction particularly radial NI (De Carvalho *et al*, 2019). In this study, our findings revealed how HUC-MSCs therapy significantly restored the combined severe radial NI. Based on clinical examination, we showed that there no complications, muscle atrophies and lameness suggesting that radial NI was treated successfully during the period of study which continued to 16 weeks. Many studies demonstrated that HUC-MSCs are capable to differentiate into osteoblasts, neurons, and endothelial cells; and have been proven to be able to promote angiogenesis, accelerate the

neurological functional recovery and bone fracture healing in animal models (Weiss *et al,* 2006; Yang *et al,* 2008; Liao *et al*, 2009). Besides, 14 different neurotropic factors related to enhance NI can be secreted by HUC-MSCs which stimulate neuronal survival, vascularization, up regulation of cell binding integrin's, delivery of anti-inflammatory molecules, and increased survival and proliferation of Schwann cells. All of these studies combined with the unique features of HUC-MSCs suggesting their potential application for the treatment of peripheral NI such as radial nerve (Guo *et al*, 2015; Ma *et al*, 2019). However, enforced rest, NSAID treatment, and plaster cast are all of value because of their roles to restore the use of the forelimb quickly (Olaifa, 2018).

Concerning to immune biomarkers of study dogs, the results experimental 1stG were revealed on significant elevation in IgG and TNF-á and significant reduction in IL-10 concentration at the 4th and 6th weeks of current study. These findings correspondent to that detected previously by (Bernstein *et al*, 1987), who detected that IgG response begins at about 30 days after NI and peaks at about 39 days. Several studies detected immune-modulatory role of IgG in many neaurological diseases (Stangel and Pul, 2006; Arumugam *et al,* 2007; Sorensen, 2008). It has been found that IgG can attenuate the effects of inflammation-mediated damage through reduction the level of pro-inflammatory cytokines and chemokines and improving neurobehavioral recovery (Nguyen *et al*, 2012). TNF-á, as a biomarker, is used frequently to provide valuable information regarding systemic inflammation response. TNF-á that produced mainly by activated macrophages and Schwann cells, can play a role in antibacterial immunity and is essential mediator of inflammation (Wagner and Myers, 1996; Song *et al*, 2012; Al-Assadi *et al*, 2018). Animal models of neuropathic pain based on various types of NI have persistently implicated a provital role for TNF-á at both peripheral and central levels of sensitization (Leung and Cahill, 2010). However, the role of TNF-á in peripheral NI has been thoroughly investigated both *in vivo* and *in vitro* by many studies that reported different outcomes. In one study, it has been suggested that TNF-á is toxic to neurons and glia (Scherbel *et al*, 1999). In another, it has confirmed that the injection of TNF-á in sciatic nerve resulted in significant NI and infiltration of inflammatory cells (Uncini and Collegues, 1999). In contrast, other studies reported that TNF-á can prevent cell death in vitro after exposure of neurons to â-amyloid peptide and in vivo after administration of excitotoxins, peripheral NI and cerebral ischemia (Barger *et al*, 1995; Bruce *et al*, 1996; Long-En *et al*, 1996). TNF-á has also been associated with the regulation of tissue remodeling, gliosis, and scar formation (Gordon *et al,* 1992; Fleur *et al,* 1996; Lindner *et al*, 1997). Liefner and Collegues (2000) demonstrated that deficient TNF-á resulted in poor macrophage recruitment and delayed in myelin removal, and thus supporting the hypothesis of direct chemotactic effect of TNF-á (Fregnan *et al*, 2012). Additionally, TNF-á down-regulates the tyrosine kinase activity of the insulin receptor, thereby increasing insulin resistance (Van Exel *et al,* 2002). Immune cells including T and B cells are important in attenuating neuro-inflammation via the modulation of various cytokines and chemokines, with IL-10 playing a central immuno-modulatory role (Liesz *et al*, 2013; Offner *et al,* 2013). In addition to macrophage, IL-10 is released by other cell types including mesenchymal stem cells (MSCs). However, severe NI can create a strong inflammatory response where production of inflammatory cytokines and recruitment of immune cells occurs rapidly, peaks early after injury (6-8 hours) and can last for weeks after NI (Kline *et al*, 2002; Garcia *et al*, 2017). Significant reduction in IL-10 detected in current study may reflect problems with vasculature including vascular and endothelial damage from inflammation and increased reactive oxygen species (ROS). Also, it detected that low IL-10 production capacity is associated with the high glucose plasma, type 2 diabetes and dyslipidemia (Van Exel *et al*, 2002; Straczkowski *et al*, 2005).

Various pathogenic factors such as infection and tissue injury can induce inflammation by causing tissue damage. In response to tissue damage, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage (Jabbour *et al*, 2009; Chen *et al*, 2018). For hematology, our findings found that total WBCs in 1st G were increased significantly and lasted for up to at least 4 weeks (2nd and 4th weeks) opposed to 3rd G. This was predominantly accounted for by an increase in the circulating lymphocytes and monocytes but not neutrophils. These results were compatible to those detected by You and Jeong (2007) and in contrast to Xue *et al* (2011), who detected that the blood parameters were within the normal range and there was only a slight increase in number of WBCs. Keskil *et al* (1994) suggested that the blood leukocytes could be a significant parameter of severity injury with prognostic value for patients with traumatic NI. Missori *et al* (1997) indicated that the neurotrauma is associated with leukocytosis. Rovlias and Kotsou (2001) have demonstrated that the level of leukocytosis is correlated directly with the severity of traumatic NI. Javdani *et al* (2018) demonstrated the

role of lymphocytes in NI and chronic pain, and suggested that the infiltration of immune cells to site of injury can helps in therapy. The absence of serum neutrophilia or neutropenia in current study might be related with the time of sample collection and analysis. Perkins and Tracey (2000) detected that the number of endoneurial neutrophils was significantly elevated only at the site of NI with a significant depletion in circulating neutrophils. These findings supported the hypothesis that a neuroimmune interaction occurs as a result of peripheral NI and is important in the subsequent development of neuropathic pain. However, high levels of neutrophils reported to potentiate the extent of NI by producing ROS and reactive nitrogen species (RNS) that can damage proteins, DNA, and lipids, and increase the extent of the inflammatory response by producing pro-inflammatory mediators such as TNF-á (Kumar *et al*, 2016; Nguyen *et al*, 2017; Zhang *et al*, 2018). In radial NI, infiltrating monocyte-derived macrophage, which uses the chemokine receptor to gain entry to injured tissues from the bloodstream are purportedly necessary for efficient Wallerian degeneration, an essential preparatory stage to the process of axonal regeneration (Lindborg *et al*, 2017). In addition, macrophage can play an indispensible role in radial NI by clearing debris and regulating the microenvironment to allow for efficient regeneration. In microenvironment, macrophage interacts with several cells to support their function most notably the Schwann cells, and glial cells of peripheral nervous system (Stratton and Shah, 2016). Kumar *et al* (2016) reported that the Schwann cells do not secrete high levels of cytokines but are potent inducers of macrophages that promote axonal outgrowth. The mechanisms governing these differences are not well understood, but it is clear that macrophages are key mediators of repair in all tissue types (Liu *et al*, 2019). In contrast to central nerves, peripheral nerves as radial nerve strong regenerative capacity and macrophages play a core role in their repair; therefore, many authors prefer to focus primarily on macrophage-mediated repair in the peripheral NI setting (Rotshenker, 2011; Cattin *et al*, 2015). After NI, especially severe and long-distance NI, local hypoxia and tissue necrosis secondary to inflammation are major obstacles for nerve repair and generation, which require a good microenvironment that is clean of necrotic tissue fragments, to promote angiogenesis, and the proliferation and migration of glial/Schwann cells (Cattin *et al*, 2015). However, macrophages and HUC-MSCs have the capacity to improve the regeneration of peripheral nerve structure and function after injury might be through providing the suitable microenvironment for macrophages and Schwann cell proliferation (Ma *et al*, 2019; Margiana *et al,* 2019).

CONCLUSION

The positive outcome achieved in this study suggesting therapeutic effects of HUC-MSCs in radial NI, and offers great promise for some disease treatment. However, the study focused on clinical results in addition to some immunological biomarkers and hematological parameters; therefore, more studies are needed to explore the mechanisms involved in the significant therapeutic effects, and to investigate the safety and efficacy of HUC-MSCs transplantation.

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Conflict of interest

There no conflict to interest.

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