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Prominent Reaction to Tissue Factor Antibody in Hemal Node of Egyptian Water Buffalo (*Bubalus bubalis*)

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ABSTRACT

Tissue Factor (TF) histology is an essential prognosticator of hemostasis and outcomes in local thrombus. Fresh tissue testing for pathologist evaluation is the best loyal technique for histology categorization, in spite of this, no literature in histology allude to the characteristics and the distribution of TF in hemal node of water buffalo species. In this research paper, author proposes an original approach to predicting cellular allocation to TF antibody in hemal node. Over the branches of iliac arteries of the abdominal aorta and superficial temporal arteries of temporal region, samples were validated on a dataset comprising 7-12 fresh hemal nodes per healthy water buffalo. Samples were fixed in 10% neutral buffered formalin, sections were organized and stained for routine histological interpretations, and anti-TF antibody was utilized for immunofluorescence examination. Hemal node held a small peanut size. It was bordered with a connective tissue capsule fashioned up chiefly of collagen fibers along with few reticular fibers, and smooth muscle cells. Inside hemal node parenchyma, lymphoid follicles and blood sinusoids were spontaneously scattered. Immunofluorescence staining highlighted the positive TF expressions to a population of cells homing the capsule, trabeculae, and blood sinusoids. Whereas a few positive TF expression remained demarking the bounds of lymphoid follicles. Distribution of positive TF cells gained a significant area % between all parts of hemal node. Hopefully, this research will provide a helpful reference for diagnosticians to monitor any morphological alterations in hemal node structure and TF locality.

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Introduction

Hemal nodes are independent secondary lymphoid organs encountered inside the blood circulation of several mammalian species as well as some birds (Zidan and Pabst, 2010). Microscopic examination of recent kinds of literature detailed that fibromuscular capsule encircles the hemal nodes externally, beneath its lymphoid rim, blood-filled subcapsular and central sinuses are sited. From the hilus, the trabecular artery within the trabeculae extends from the capsule to the interior part (Artemeva, 2018). Several researchers confirmed that hemal nodes parenchyma comprise of a cortex and medulla in sheep and dromedary camel (Zidan and Pabst,2004), whereas other investigators haven't distinguished these areas in hemal nodes of roe deer and water buffalo (Akaydin and Kabak, 2010; Zidan and Pabst, 2010). Parenchymal constituents are organized as lymphoid follicles, lymphatic cords, and blood sinusoids. The quantity and histological features of hemal nodes vary greatly in various ruminant species. There are two forms of lymph nodules: primary and secondary. Primary lymph nodules encompass only the small B lymphocytes whereas secondary lymph nodules possess a variety of cells in a light germinal center as T lymphocytes, macrophages, encircled by a darker mantle of small B lymphocytes (Ceccarelli *et al*., 1986). All organ gains its support from a well net of diffuse lymph reticular tissue and smooth muscle cells scattered in between (Artemeva, 2018). They exhibit critical protection against blood-borne infection and the clearance of damaged and aging erythrocytes through their blood sinusoids and the close contact among immune cells and the blood. Therefore, they can yield similar hematological and immunological roles of the spleen (Zidan and Pabst, 2010). Even though, they are mistreated by most immunologists.

The earliest extrinsic coagulation technique depends on four factors: fibrinogen (Factor I (FI)), prothrombin (Factor II (FII)), thromboplastin (Factor III (FIII) or Tissue Factor (TF)), and Ca++ (Factor IV (FIV)) (Grover and Mackman, 2018). Tissue factor (TF), is an integral membrane glycoprotein that is crucial for life. It is recognized as the principal cellular motivator for coagulation protease cascade and stimulator for protease-activated receptors (PARs) (Mackman, 2004). On the cell surface, TF serves in two ways; one as a receptor with high affinity properties and the other as a cofactor for factors VII and VIIa, which in turn will trigger the activation of factors IX and X. The

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outcome will associate with local thrombogenesis, fibrinogenesis, and platelet aggregation (Grover and Mackman, 2018).

The TF-FVIIa complex is critical for hemostasis and affords extra protection to vital organs, for instance, the heart, lung, and brain. TF contributes to arterial and venous thrombosis. TF also plays a leading role in the formation of thrombi after rupturing of atherosclerotic plaques. Suppression of all resources of TF is combined with intensified bleeding. However, targeted inhibition of pathological TF expression, for instance in monocytes, may reduce thrombosis associated with different diseases (Grover and Mackman, 2018). More studies are required to recognize the cell types that express TF in diverse tissues and regulate hemostasis in basal and pathological situations.

To the best of the author knowledge, no kinds of literature are found concerning TF in hemal nodes, as a result, this paper examined specific monoclonal antibody of buffalo tissue factor on the hemal node of water buffalo species. The fundamental role of TF expression in normal tissue is to be as a reference in monitoring any morphological changes in their location and elucidating the pathophysiology of life-threatening thrombotic cases.

Materials and methods

Ethical approval

All appropriate international, national, and/or institutional regulations for the care and use of animals were ensued. Ethical approval no. CU II F C 123 18 from the Institutional Animal Care and Use Committee (IACUC).

Sample Collection

Selection of samples in this work was based on animals who look apparently healthy and did not suffer from any diseases. The water buffaloes were subjected to Islamic slaughtering for human consumption in a Munib slaughterhouse, Giza, Egypt. Fresh specimen of hemal nodes were gathered from six adult water buffaloes (n=6), both sexes (3 females, 3 males), and 500± 50 Kg. Roughly 7-12 hemal nodes were assembled from each buffalo all around the iliac arteries of the abdominal aorta and arterial branches of temporal region for gross and microscopic examination. Immediately after getting samples, hemal nodes were fixed in 10% neutral buffered formalin and transported in icebox to the histology lab, Faculty of Veterinary medicine, Cairo University, Giza, Egypt.

Histological Examination

Samples treated for dehydrating using grading ethanol, xylol solutions and for embedding by paraffin. Thin sections of paraffin (5-6 μ) were obtained using a rotatory microtome and stained with hematoxylin and eosin (H&E).

Immunofluorescence Staining Protocol

Mounted slides with hemal nodes samples were treated for immunofluorescence identification of tissue factor using the following protocol: The slides were kept in an oven at 60 °C for 20 min. Each slide was deparaffinized by washing with 100% xylol twice for 15 min. The slides were gradually rehydrated by immersion twice in ethanol (100%), 5 min/each, then once in ethanol (90%, 70%, 50% and 30%), then distilled water for 5 min/each. The slides were incubated in a preheated Daco® citrate buffer for 20 min to retrieve the antigens, then allowed to cool to room temperature. The slides were washed three times for 3 min with 0.05% Tween in phosphate-buffered saline (PBS). Tissue sections were marked with a Daco® pen and fixed by adding twenty ≒L of 4% paraformaldehyde in PBS at pH 7.4 for 30 min using a darkened and humidified chamber. The slides were washed again with 0.05% Tween/PBS 3 times for 3 min/each. Tissue sections were blocked by adding twenty ≒L of a blocking solution of 1% BSA, 10% horse serum and 1% PBS for 1 h at room temperature, using a darkened humidified chamber. The slides were washed again with 0.05% Tween/PBS 3 times for 3 min/each. The slides were incubated for 3 hours at 37 °C followed by overnight incubation at 4 °C with the appropriate primary antibody; Anti-Bovine Transferrin, FITC, Polyclonal Antibody (rabbit IgG), NCBI GI #339453, NCBI GeneID#7018, NCBI Accession #AAA61140.1, Molecular Weight: 77,064 Da (Biotechnology, San Diego, California, United States). The slides were washed again with 0.05% Tween/PBS 3 times for 3 min/each. The slides were incubated for 30 min at 37 °C with a goat anti-rabbit secondary antibody (Texas Red, Alexa Fluor 488) and rabbit anti-rat (Texas red) (Abcam Cambridge, MA, USA).

The slides were washed again with 0.05% Tween/PBS 3 times for 3 min/each. The slides were incubated for 5 min at 37 °C with 4′,6-diamidino-2-phenylindole; (Abcam Cambridge, MA, USA) for nuclear staining. The slides were washed again with 0.05% Tween/PBS 3 times for 3 min/each, then dried and mounted with cover slips using Fluoromount® mounting solution (Abcam Cambridge, MA, USA) (Vollmar *et al*., 1998).

Tissue sections were examined and imaged using a Nikon fluorescence microscope (Eclipse 90i with a DS-U3 imaging system, Nikon Metrology, Inc., USA) under blue and green channels at the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Evaluation of immunofluorescence results by count percentage (count of antibody/specific area)

Cell numbers were evaluated as the quantity of cells for each area by means of the cell-counter and color-threshold for ImageJ and the outcomes were handled to determine count percentage (count %) of TF antibody in all lymph nodule, peripheral area of lymph nodule, central area of lymph nodule, lymph reticulat tissue, and blood sinusoids of hemal node compartments.

Statstical Analysis

Data associated to count % of TF antibody existing as mean and standard error "SE". Test of Homogeneity of Variances, Anova analysis between groups, Post Hock Test, and Tuckey HSD were assessed by means of SPSS for estimating P-Value. In statistics, P-Values that is fewer than 0.5 raised for a significant result.

Results

Macroscopic observations in this research highlighted the absence of hemal nodes around branches of superficial temporal artery; cornual and palpebral arteries in temporal region of water buffalo species (Figs. 1a, 1b). In opposing, hemal nodes existed as a deep brown organ dispersing in between adipose tissue along iliac arteries of the abdominal aorta (Fig. 2a). It held a size varied from small pinhead to large peanut. Approximately the major quantity of hemal nodes are in small size shape (Fig. 2b).

Histological findings revealed that hemal node is a compact organ encompassed connective tissue stroma and cellular parenchyma. Stroma is organized as fibromuscular connective tissue capsule consisting of collagen fibers, elastic fibers, smooth muscle cells and few reticular fibers. Connective tissue

trabeculae are extended from capsule to enter the hemal node parenchyma. Endothelial cells outlined the subcapsular sinuses and trabecular sinuses (Fig. 3a).

On the other hand, the parenchyma of hemal node in water buffalo constructed of lymphoid follicles, lymphatic cords, and blood sinusoids. The organization of these components was fashioned in two different forms. Hemal nodes assembled as lymphoid follicles in cortex peripherally and lymphatic cords as well as blood sinusoids in medulla centrally (Fig. 3b).

The parenchyma was reinforced by a fit web of reticular cells and fibers which designed a basic backbone. Lymphoid follicles were round structures homing lymphocytes, follicular dendritic cells, and plasma cells (Figs. 4a, 5a).

Tissue factor antibody represented with a difference expression along hemal node of water buffalo. All expressions situated inside the cell. On the level of lymph nodule, tissue factor antibody highlighted a positive expression on cells bordering the external outlines of lymph nodule forming circle, in opposite to cells homing the internal part responded with a negative reaction. Majority of cells in blood sinusoids expressed a positive reaction to tissue factor while small ones got a negative appearance. Few scattered positive cells to tissue factor were noticed among the cellular population of lymph reticular tissue. All cells lining the internal surface of branched arteriole found its positivity to tissue factor antibody (Fig. 4b).

In some hemal nodes of water buffalo, the TF+ cells in

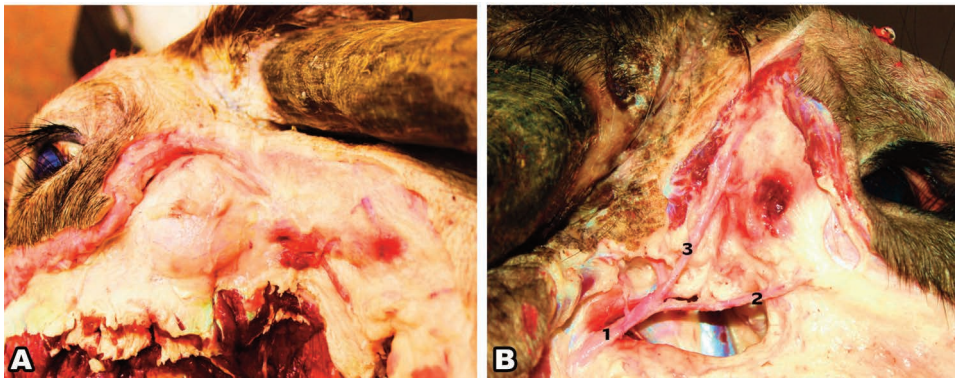

Fig. 1. (A) Photograph presenting the anatomical dissection of temporal region in water buffalo lacking hemal node organ. (B) Photograph showing the arterial branches of superficial temporal artery (1); palpebral arteries (2) and cornual arteries (3).

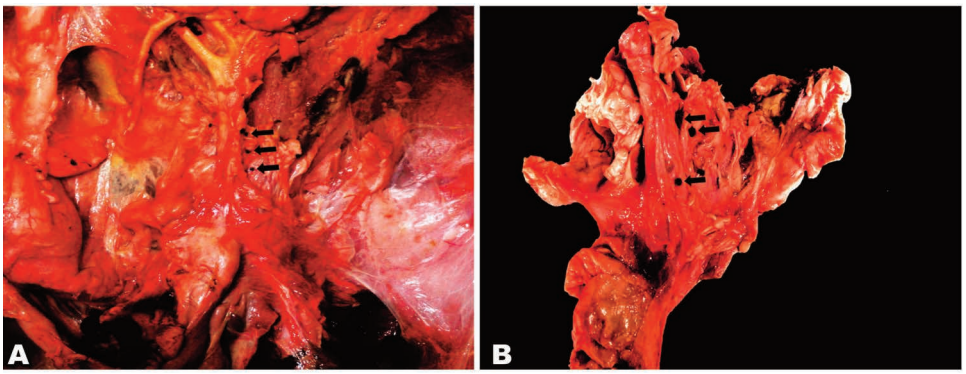

Fig. 2. (A) Photograph of hemal nodes in water buffalo species as a deep brown structure scattering in between adipose tissue lengthwise iliac arteries of the abdominal aorta. (B) Photograph giving an approximate size of hemal node from a small pinhead to large peanut.

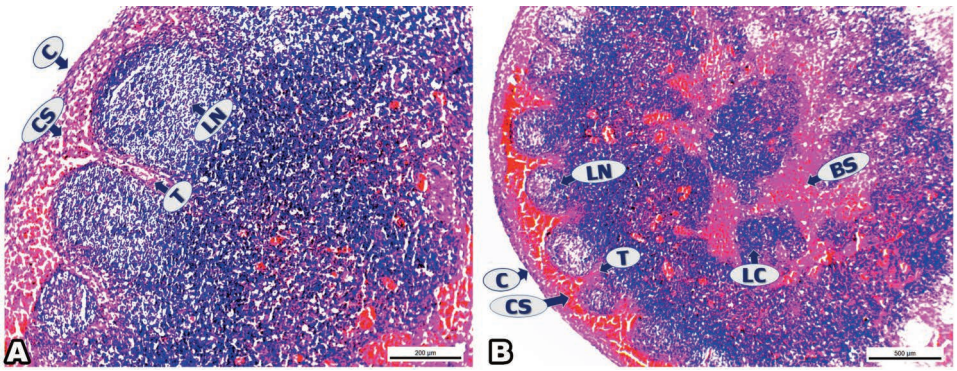

Fig. 3. (A) Photomicrograph of hemal nodes in water buffalo highlighted the connective tissue capsule (C), subcapsular sinus (CS), trabeculae, and trabecular sinus (T). Notice the round structure of lymph nodule (LN) (H&E x100). (B) Photomicrograph demonstrated the distribution of lymph nodules (LN), blood sinusoids (BS), and lymphatic cords (LC) in hemal node of water buffalo. Also observe the capsule (C), subcapsular sinus (CS), trabeculae, and trabecular sinus (T) (H&E x40).

lymph nodules didn't form a circle in the external border but arranged as scattered positive cells in the same area (Fig. 5b).

Lymphatic cords are irregular cords comprised of mostly lymphocytes. The lymphatic cords were detached by large, great, asymmetrical blood sinusoids (Fig. 6a). Dimension of these sinusoids was designed to be variable, enveloped blood vessels inside it and supported by myofibroblast cells (Figs. 6a, 7a). Majority of cells responded to tissue factor antibody in this area represented as blood sinusoids, lining of enveloped blood vessels, and myofibroblast cells. In comparable to the pervious results, cells comprising lymphatic cords maintained negative except small intermittent cells got a positive expression (Figs. 6b, 7b, 7c).

The stastical analysis of positive tissue facor cells confirmed a significant differences between all parts of water buffalo hemal node. Data are shown in Table 1 and Fig. 8.

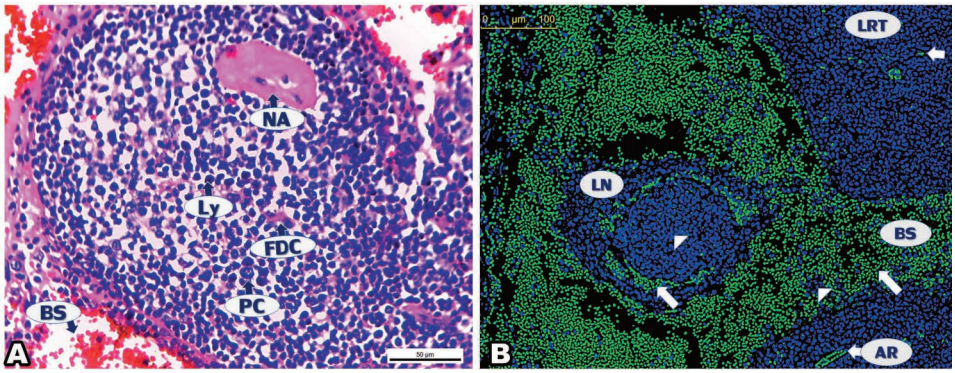

Fig. 4. (A) Photomicrograph spotted on lymph nodule in hemal nodes of water buffalo. It is organized in a round aggregations of lymphocytes (Ly), follicular dendritic cells (FDC), plasma cells (PC) and supplied by nodular artery (NA). Notice the surrounding blood sinusoid (BS) (H&E x400). (B) Photomicrograph remarked immunoreactivity of tissue factor antibody in water buffalo hemal node. Inside lymph nodule (LN), expression detected as peripheral circle of positive TF+ cells (arrow) in contrast to internal negative cells (arrowhead). Majority of cells in blood sinusoids (BS) expressed a positive clusters (arrow) opposite to few ones got a negative appearance (arrowhead). Sight the speckled positive cells to tissue factor inside lymph reticular tissue (LRT) and cells lining the branched arteriole (AR) (TF x100).

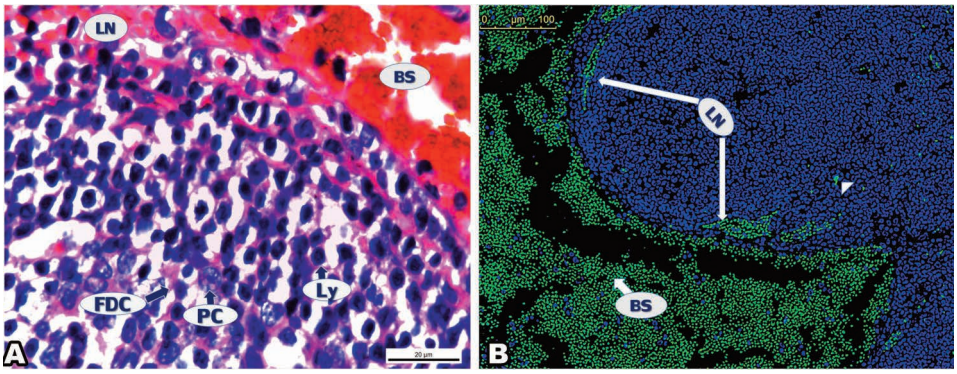

Fig. 5. (A) Photomicrograph demonstrated the lymph nodule (LN) in hemal nodes of water buffalo. It is structured in a round masses of lymphocytes (Ly), follicular dendritic cells (FDC), and plasma cells (PC). Notice the surrounding blood sinusoid (BS) (H&E x1000). (B) Photomicrograph determined scattered positive cells in the peripheral part of lymph nodules (Arrows). See the adjacent blood sinusoid (BS) (TF x100).

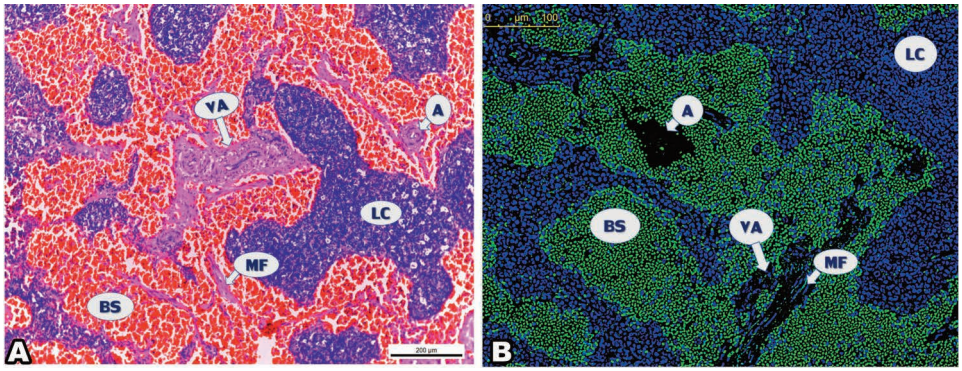

Fig. 6. (A) Photomicrograph presented the center region inside hemal node of water buffalo. It arranged as irregular lymphatic cords (LC) which separated by blood sinusoids (BS). Inside blood sinusoid (BS), vascularized area (VA), branched artery (A), myofibroblast cells (MF) are situated (H&E x100). (B) Photomicrograph exhibited the reactivity of hemal node cells to tissue factor antibody beyond blood sinusoid (BS), vascularized area (VA), branched artery (A), myofibroblast cells (MF), and lymphatic cords (LC) (TF x100).

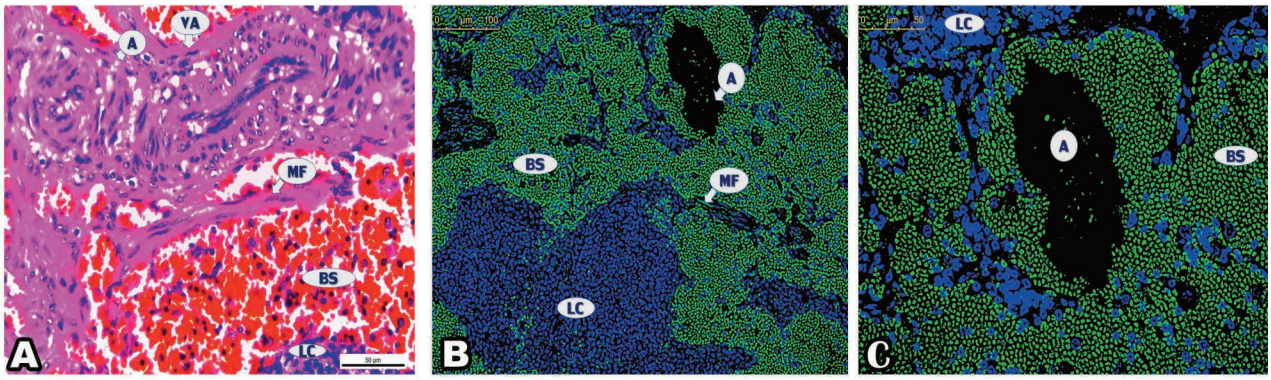

Fig. 7. (A) Photomicrograph detailed the vascularized area (VA) inside hemal node of water buffalo. It entailed blood sinusoids (BS) homing branched artery (A) and myofibroblast cells (MF) encircling lymphatic cords (LC) (H&E x400). (B, C) Photomicrograph marked the expression of hemal node cells to tissue factor antibody through blood sinusoids (BS), lining of enveloped blood vessels (A), myofibroblast cells (MF), and lymphatic cords (LC) (TF x100 - x50).

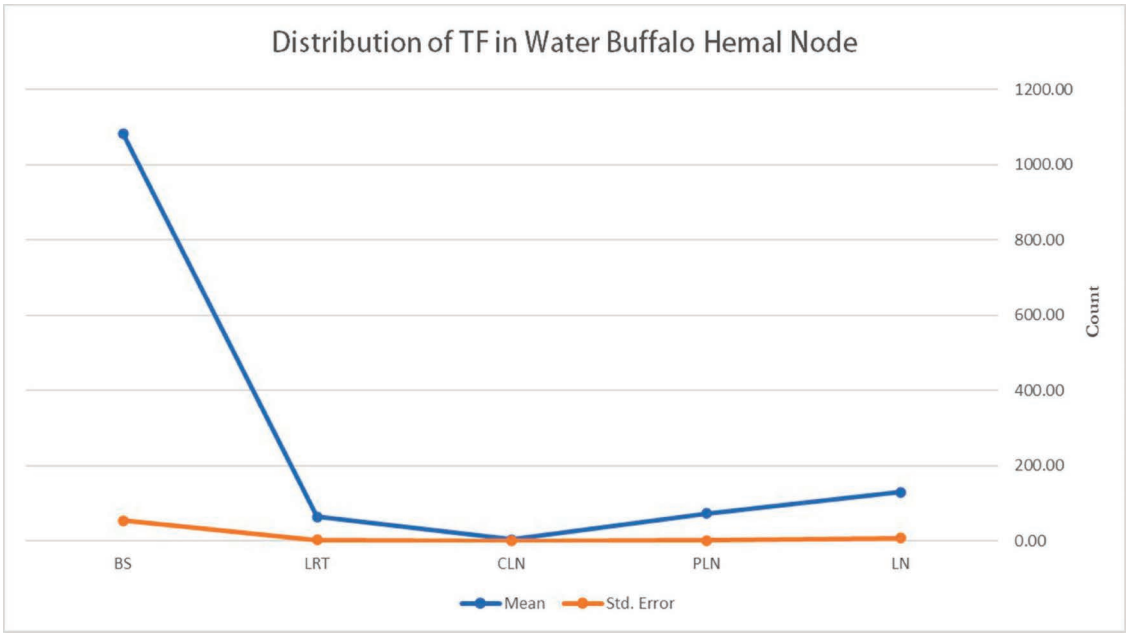

Fig. 8. Tissue factor count (Mean value) in Water Buffalo Hemal Node. LN: all lymph nodule area; PLN: peripheral area of lymph nodule; CLN: center area of lymph nodule; LRT: area of lymph reticular tissue; BS: Blood Sinusoid area.

Table 1. Distribution of TF in water buffalo hemal node

Area	Mean ± SE	*P*- Value
LN	130.17±8.24^{b}	
PLN	73.67±1.23^{a}	
CLN	3.67±0.88	.000**
LRT	64.17±3.42	
BS	1082.0±54.92^{c}	

TF=Tissue Factor, LN= Lymph Nodule, PLN= Peripheral Lymph Nodule, CLN= Center Lymph Nodule, LRT= Lymphreticular Tissue, BS= Blood Sinsoid. Different superscripts indicate significant difference at P ≤ 0.05. **P-Values ≤ 0.001 are highly significant.

Discussion

Such research is, as far as we know, the first to explore the distribution of tissue factor throughout cells of hemal node and to evaluate its critical effect in organ stability. Previous reports have assessed the normal structure of hemal node, but the distribution and importance of tissue factor in this organ was not investigated. Author of this study covered the topic from three main themes: macroscopic gross anatomy, light microscopic examination, and immunofluorescence aspect.

This investigation in the anatomical parameters of water buffalo hemal nodes emphasized their absence around branches of superficial temporal artery; cornual and palpebral arteries in temporal region. Although many studies detected hemal nodes in the head region of ruminants (Bacha and Bacha, 2000; Snider *et al*., 2003), Casteleyn *et al.* (2008) alluded that in 62 cattle, only 89% had hemal node in their temporal regions which give a reason to their absence probability in this study.

On the other hand, hemal nodes in this research presented along iliac arteries of the abdominal aorta as a deep brown organ dispersing in between adipose tissue. They hold a variety of sizes from small pinhead to large peanut. Roughly the main size of hemal nodes are in small size shape. These results simulated the data documented by Kannan *et al.* (2019) in Indian buffaloes. Accordingly, the quantity and distribution of hemal nodes is unusual and species specific.

Relating to the histological features of hemal nodes, they fluctuate substantially in various ruminant species. Histological findings in this research revealed that hemal node is a compact organ encompassed connective tissue stroma and cellular parenchyma. Stroma is organized as fibromuscular connective tissue capsule consisting of collagen fibers, elastic fibers,

smooth muscle cells and few reticular fibers. These findings meet the results obtained by Zidan and Pabst (2010) and Kannan *et al*. (2019) in ruminant. The existence of smooth muscle cells in between fibrous capsule of buffalo hemal node may refer to its contractile ability, storage functions, on top of its capability to tolerate severe anemic conditions.

From capsule, connective tissue trabeculae are extended to enter the hemal node parenchyma. The author of this study suggested that the observed trabeculae provide elasticity, contractility, and serving a rigid framework. The capsule enveloping area of water buffalo hemal node is supplied by two types of sinuses: subcapsular sinuses under hemal node capsule and trabecular sinus around trabeculae. Similar findings were reported in different animals as what was obtained in water deer by Akaydin and Kabak (2010). These sinuses may be involved in erythrophagocytosis and lymphocyte transport.

Concerning hemal node parenchyma in this work, it assembled as lymphoid follicles in cortex peripherally and lymphatic cords as well as blood sinusoids in medulla centrally. It comes hand to hand with the records found in sheep and dromedary camel (Zidan and Pabst, 2004). On the contrary, these regions were not distinguished in hemal nodes of roe deer (Akaydin and Kabak, 2010). This histological diversity among animals comes through the degree of roles that this organ participates in as per the authors recommended.

All structures of hemal node parenchyma in the present investigation gained their support by an appropriate net of reticular cells and fibers which intended a chief mainstay as documented by Kannan *et al*. (2019). Moreover, Gargiulo *et al*. (1987) clarified that this reticular meshwork aid in the migration of large numbers of free blood cells to accumulate in the trabecular and subcapsular sinus.

Regarding the reactivity of tissue factor antibody in this study, it corresponded with a different expression along the hemal node of water buffalo. This normal functional expression of tissue factor in hemal node tissue will illuminate its procoagulant and biological functions which can be classified into two main roles: hemostatic and non-hemostatic. The first presented in its ability to maintain hemostasis in all body tissues while the later characterized by its involvement in vital biological processes like; cell migration, angiogenesis, inflammation, metastasis, vascular development, and adaptive immunity. This exploration found evidence for scientist's consent that tissue factor cell type-specific distribution offers a hemostatic envelope to limit bleeding after vessel injury. In accordance with this, a prominent level of tissue factor in some organs than others will afford extra hemostatic safeguard to these vital organs (Mackman, 2004).

Another promising finding was that all expressions of tissue factor located inside cells. Equally as detected by Drake *et al*. (1989) who said that tissue factor expressions seem to be cell linked and diffuse instead than crucial or restricted to the cell membrane. However, Mackman (2004) clarified the critical role of tissue factor in hemostatic safety and structural preservation of the vasculature needs its extracellular position than its cytoplasmic location. In spite of this, later investigations revealed that some TF has no procoagulant activity (Böing *et al*., 2009) but that it may well defend cells from apoptosis (Boltzen *et al*., 2012) and stimulate tumor progression and angiogenesis (Godby *et al*., 2012).

Extensive results carried out display that lymphoid follicles appeared as round structures homing group of cells like lymphocytes, follicular dendritic cells, and plasma cells. Tissue factor antibody highlighted in this area as a positive expression in cells bordering the external outlines of lymph nodule forming either circle or scattered cells in the same area, in opposite to cells homing the internal part responded with a negative reaction. This is consistent with data recorded by Mackman (2004) and according to what has been found in previous results of Zidan and Pabst (2004) in dromedary camel, Akaydın *et al*. (2018) in deer, Bozkurt *et al*. (2018) in goat, and Kannan *et al*. (2019) in Indian buffalos, the cells responded to tissue factor antibody in this study, are mainly of T lymphocyte type as they occupied the margin of the follicles, indistinct to the low quantity of T lymphocytes presented inside follicles. Besides, Ruf and Riewald (2013) proved that T cells can participate to the thrombotic progression through their representation of TF. At odds with Casteleyn *et al*. (2008) who stand out against these findings and revealed a greater quantity of T-lymphocytes inside lymphoid follicles.

A further novel finding in a hemal node of water buffalo is that majority of cells in blood sinusoids expressed a positive reaction to tissue factor while small ones got a negative appearance. Few scattered positive cells to tissue factor were also noticed among the cellular population of lymph reticular tissue. This non-standardized allocation of tissue factor expression through the hemal node reinforces that tissue factor expression is controlled by various cell types and at the same time may be related to some variation between species as suggested by Drake *et al*. (1989). In view of that, when comparing these results to those of older studies, it must be pointed out that tissue factor disperses in a non-consistent way in endothelial cells, fibroblast, follicular dendritic cells, and trabeculae cells (Drake *et al*., 1989; Mackman, 2004).

Posing attention to the response of vascular endothelium to tissue factor antibody in water buffalo hemal node, it is hypothesized that there is a correlation between coagulation and induced expression by intravascular cells in consequence of the results obtained in all cells lining the internal surface of branched arteriole in this study which found its positivity to tissue factor antibody. Overall, these results are in accordance with findings reported by Mackman (2004) who compared the reactivity of muscular arteries and large arterioles with veins in the spleen and confirmed the intense reaction to tissue factor in the first unlike the moderate and irregular expression to the latter. The latest study testified that perivascular TF was pre-bound to FVII/FVIIa before vessel injury (Hoffman *et al*., 2007). And this preformed TF-FVIIa complex would permit fast initiation of clotting following vessel injury (Hoffman and Monroe, 2009). These data indicate that TF-reliant on thrombin production guides to fibrin sedimentation and platelet stimulation that behave co-operatively to create and alleviate the hemostatic plug (Grover and Mackman, 2018).

Consequently, the normal distribution of tissue factor in hemal node will give an explanation of the capability of the intravascular cells to activate the coagulation if vascular integrity is altered (Drake *et al*., 1989). Furthermore, it highlights the prospective effect of TF in particular uncontrolled cases like; inflammation, various infections, thrombus formation, cellular immunity (Cybulsky *et al*., 1988; Drake *et al*., 1993) and disseminated intravascular coagulation (Østerud and Bjørklid, 2001).

Lymphatic cords were highlighted in this research as irregular cords comprised of mostly lymphocytes. This result ties well with previous studies by Kannan *et al*. (2019) who added other cells like plasma cells, mast cells, erythrocytes, and macrophages were similarly detected. Additionally, most cells of lymphatic cords in this study revealed a negative reaction except small intermittent cells got a positive expression. Authors suggested that those positive cells might be macrophages respecting their key role in innate immunity, they probably accelerate clot development to restrict the propagation of pathogens (Grover and Mackman, 2018).

Extensive results conducted show that lymphatic cords were detached by large, great, asymmetrical blood sinusoids. Dimension of these sinusoids was designed to be variable, en-

veloped blood vessels inside it, and supported by myofibroblast cells to alleviate its crucial role inside the animal body. This fits the reported literature in buffaloes by Zidan and Pabst (2010) and Kannan *et al*. (2019) who discussed that though the sinusoids were noticed to be larger, storage role of blood in hemal node could not be probably owed to the minor mass of the hemal node comparative to the bodyweight of buffalo.

Majority of cells responded to tissue factor antibody in this area represented as blood sinusoids, lining of enveloped blood vessels, and myofibroblast cells. These data confirmed by previous studies which detected TF in interspersed cells between bundles of smooth muscle (Rhodin, 1980), macrophage (Drake *et al*., 1993), perivascular cells, such as adventitial fibroblasts and pericytes (Bouchard *et al*., 1997), endothelial cells (Szotowski *et al*., 2005), neutrophils, eosinophils, T-cells (Uderhardt etal., 2017), megakaryocyte and platelets (Grover and Mackman, 2018). Others failed to identify TF expression in neutrophils or eosinophils (Sovershaev *et al*., 2008), platelets (Østerud and Olsen, 2013), and incredibly low expression in endothelial cells (Antoniak and Mackman, 2017).

Activation of TF or defects occurred in TF expression provokes pathological coagulation that generates inflammation, hemostatic, and life-threatening thrombotic diseases (Drake *et al*., 1989). TF engages in the pathogenesis of both arterial and venous thrombosis (Owens and Mackman, 2010) as within atherosclerotic plaques (Asada *et al*., 1998) and TF-positive micro vesicles in tumors (Grover and Mackman, 2018). TF-positive platelets correlated to cases of acute coronary syndromes and diabetes mellitus (Gerrits *et al*., 2010) and they donate to thrombosis relatively than hemostasis (Mackman and Luther, 2013). TF induced on endothelial cells promotes the stimulation of coagulation in sepsis (Grover and Mackman, 2018). Complete deficiencies of TF in mice lead to universal death during embryonic development or shortly after birth (Dewerchin *et al*., 2000). Based on that, maintaining the stability and reactivity of hemostatic system is vital to normal physiological necessity and reduces the possible incidence of intravascular coagulation (Drake *et al*., 1989).

Conclusion

The current study investigated one of transmembrane proteins in water buffalo hemal nodes to determine its distribution in hemal node tissue. Results confirmed the principal location of TF in blood sinusoids versus its scarce inside lymphnodules. These findings will direct the efforts to understand the pathobiology of a variety of ruminant diseases and improve our ability to diagnose and treat such diseases.

Conflict of interest

The author declare that she has no known competing financial interest or personal relationship that could has appeared to influence the work reported in this paper.

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