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Original Article:

Qualitative Study on Endothelial Cell–to–cell–junction Disassembly in Severe Burn Injury

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Abstract

Introduction. Endothelial gap in severe burn injury remain a mystery. Capillary leaks possess its own characteristics, which is found in burned– and non–burned area. The gaps remain up to 10 post burned days or more. This is somehow representing the feature of systemic capillary leaks syndrome at the first date. VE–cadherin of adherens endothelial junction molecules known to be temporarily disassembled following thermal exposure, but there's a question about reversibility. Question is also addressed to occludin of the tight junction molecules. We run a study to investigate these junction molecules.

Method. We run an investigation to find out both molecules qualitatively descriptive on 30 burn patients enrolled, consist of 20 severe– and 10 of non–severe burn. Samples of moderate size vein taken from burned– and non–burned area were subjected to study of histomorphology and immunohistochemistry. Light microscopic study and polymerase chain reaction test were carried out to compare the features and its expression. Analysis is carried out to find the difference, specificity and sensitivity.

Results. Samples took within the first 8 hours following ER presentation showed severely deteriorated endothelial lining and both of VE–cadherin and occludin dissociation. This endothelial junction disassembly was found in both of burned– and non–burned area; both of severe– and non–severe burn as well. In burned area, mRNA expression of VE–cadherin found to be increased, as occludin decreased. In severe burned group, mRNA expression of VE–cadherin as well as occludin found to be increased. VE–cadherin synthesis was found to be earlier than occludin.

Conclusion. Dissociation of both of endothelial cell–to–cell molecules junction show no differences between the two groups, and between burned– and non–burned areas.

Keywords: *endothelial junction disassembly; severe burn injury*

Introduction

It is postulated that edema following burn injury due to increased capillary permeability is a natural process. Such a postulate has been established for a long time, and each clinician believes that edema manifests in minutes to hours following injury is influenced by histamine as the early inflammatory mediator released soon after a thermal exposure. It reached its peak level as much as 8–12 hours, and then subsides as histamine level decreased. The capillary permeability returns to normal as histamine's role lasts in 72 hours' post burned.[1] This has led to such a concept and the way of thinking describing that intravascular volume depletion due to capillary leaks is the main problem during the first 48 hours following burn injury.[2,3] In daily clinical practice, we found it different, particularly in severe burns. In burns case, more than 25% total body surface area (TBSA), the edema might be found as much as 7–10 post burned days. Massive edema was found both in burned– and non–burned area. Ironically, these cases were massively resuscitated using crystalloids during pre–hospital setting but found to be hypovolemic during presentation at the emergency room – as if it is under resuscitated – and, with massive edema.[4] Edema becomes progressive as the fluid administration is proceeded to correct hypovolemia; suggesting a condition of fluid creep, the phenomenon described by Saffle in his publication.[5] In this difficult situation, the administration of controversial colloid was also not helpful.[6,7]

Method

Twenty patients of severe burn and ten patients of non–severe burn who's agreed and signed the informed consent were enrolled. The samples of vessels of moderate size vein in burned area were taken during emergency procedure of escharotomy or fasciotomy, whereas vessels in non–burned area were obtained through surgical venous access as an important procedure to precede fluid resuscitation. The samples of vessels in 3 cm long were placed in sterile tubes and labeled: 1) burned area of severe burn (BSB), 2) non–burned area of severe burn (NBSB), 3) burned area of non–severe burn (BNSB), 4) non–burned area of non–severe burn (NBNSB). These samples were stored in temperature of –4ºC then prepared for study of immunohistochemistry and mRNA expression using Real Time–PCR.

Preparation for immunohistochemistry test of VE–cadherin consists of six steps, i.e. 1) former preparation, 2) rehydration, 3) incubation using primary antibody of VE–cadherin, 4) incubation using secondary antibody (biotinylated antibody), 5) incubation using tertiary antibody (Avidin, Streptavidin) and 6) sample staining using Gills III–hematoxylin. Samples preparation for immunohistochemistry of occludin consists of twelve steps, i.e. 1) former preparation, consists of deparaffinization and rehydration, 2) blocking endogenous peroxidase, 3) Pretreatment using TE, 4)

blocking background sniper, 5) incubation using primary antibody of Anti–Occludin, 6) incubation using secondary antibody (universal link), 7) incubation using tertiary antibody (TrekAvidin–HRP label), 8) incubation using diaminobenzidine (DAB), 9) sample staining using hematoxylin, 10) incubation using lithium carbonate, 11) dehydration, and 12) clearing process.

Preparation for expression of mRNA using Real Time–PCR, consists of three steps, i.e. 1) RNA extraction, 2) separation of RNA, 3) RNA isolation. Specific primers required for each mRNA expression are available in Primer–Blast National Center for Biotechnology Information (NCBI) websites. Amino acid configuration of human VE–cadherin and occludin in FASTA format is used to find out the primer using the provider's primer–blast program. Beta–actin is used as a control since beta–actin is a cytoskeleton molecule (house–keeping gen) that always is expressed. Using this control, any changes of both expression of VE–cadherin and occludin will be able to be detected. The information available from primer–blast for VE–cadherin is NM_001795.3 Homo sapiens cadherin 5, type 2 (vascular endothelium) (CDH5), mRNA. Forward primers 5'–GACGCCCGGCCTTCCC TCTA–3' (Tm 60.04, GC 70.00%) and reverse primers 5'–TCGTGGTCCGC CTCGTCCTT–3' (Tm 59.90, GC 65%). Whereas, information available from primer–blast for occludin is NM_003257.3 Homo sapiens tight junction protein 1 (zona occludens 1) (TJP1), transcript variant 1, mRNA. Forward primers 5'–AAGGCGGGGC CTACACTG AT–3' (Tm 57.45, GC 60.00%) and reverse primers 5'–TGCTG GGTTTGTTTCA GGCGA–3' (Tm 57.16, GC 52.38%). And for beta–actin (actin β) is NM_001101.3 Homo sapiens beta–actin (ACTB), mRNA. Forward primers 5'–ACAGAGCCTCGCCTTT GCC GA–3' (Tm 60.78, GC 61.90%) and reverse primers 5'–TGCCGTGCTCG ATGGGTACT–3' (Tm 59.98, GC 61.90%). Kit of iScript one–step SBYR Green (Bio–Rad) is used for this purpose.

The study for immunohistochemistry is carried out under microscope of Nikon Eclipse 80i with each of 40 and 100 times objective lens magnification. This examination is focused on distribution and intensity of fine brown granules of VE–cadherin and brown granules of occludin. In the description of molecule disintegration, the scoring system is used where the more score is the more severe (table 1).

Table 1. The scoring of endothelial changes

	Histomorphology findings	Score
A.	Adhesive molecule distributed well around attached endothelium with strong intensity	1
B.	Adhesive molecule distributed around detached endothelium with strong intensity	2
C.	Adhesive molecule distributed around detached endothelium with weak intensity	3
D.	Distribution of adhesive molecule is undetectable	4

VE–cadherin and occludin mRNA expression is measured using Real Time–PCR (Mini Opticon™ System, Bio–Rad). This is purposed to measure both of adhesive molecules of endothelial cell–to–cell junction semi–quantitatively; i.e. measuring its expression in the early phase (exponential) and not in the later phase (plateau) which is measuring the outcome. Prior to measurement using Real Time–PCR, the concentration of isolated mRNA obtained in sample preparation is calculated using spectrometer (Genesys 10 UV, Thermo Scientific) in 260 nM.

The concentration of VE–cadherin, occludin and beta–actin (of spectrometry) as well as cycle threshold (Ct) of mRNA of VE–cadherin, occludin and beta–actin (of Real Time–PCR) is documented using Microsoft Excel ver. 2007 as raw data. Statistical analysis of Ct is processed using SPSS ver.12, whereas the expression of mRNA in ratio is calculated according to the method of Livak.[11,12] It was hypothesized that the VE–cadherin and occludin, both of endothelial junction molecules of endothelial cells are disassembled in burned– and non– burned area as well. The Ethical Committee of Ethics Faculty of Medicine, Universitas Indonesia approved the study (Ethical Clearance) No. 50/PT02.FK/ETIK/2008.

Results

In the preliminary study, we found endothelial lining was severely disintegrated both in vein and capillaries, in burned– and non–burned area; both in critical– and non–critical burn as shown in fig.1.[13]

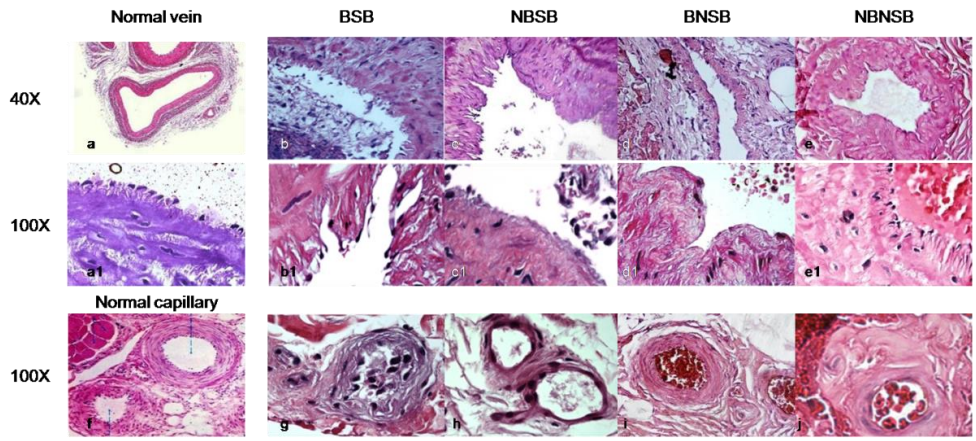

Figure 1. The histomorphology features of endothelial lining of vein and capillaries in burned– and non–burned area in both of critical– and non– critical burn using hematoxylin eosin staining. a to e in objective lens magnification of 40 times, a1 to e1 in objective lens magnification of 100 times. a. A normal vein of a normal young volunteer with a clear lumen. b. Vein of BSB. The lumen filled with detached endothelium fibrin and thrombus. c. Vein of NBSB. The lumen filled with erythrocytes or clear. d. Vein of BNSB. The lumen filled with erythrocytes or clear. e. Vein of NBNSB. The lumen filled with erythrocytes or clear. a1. A well maintained endothelial lining of a normal vein. b1. Vein of BSB is showing disintegration of endothelial lining with disrupted endothelial alignment and disconfiguration of the tunica intima. c1 to e1, i.e. vein of NBSB, vein of BNSB and vein of NBNSB are showing disintegration of endothelial lining with disrupted endothelial alignment. f. Endothelial lining of a normal capillary. g. Capillary of BSB showing occluded lumen with disintegrated endothelial lining and disruption of endothelial alignment. Disconfigured peri–capillary bed is shown and crowded with erythrocytes. h. Capillary of NBSB showing the integrity of endothelial lining and endothelial alignment is no longer maintained. Disintegrated peri–capillary bed is shown. i. Capillary of BNSB showing occluded lumen with disintegrated endothelial lining and disruption of endothelial alignment.

Disintegrated peri–capillary bed is shown. j. Capillary of NBNSB showing disintegrated endothelial lining with a maintained endothelial lining. Peri–capillary bed edema is shown. BSB: burned area of severe burn, NBSB: non–burned area of severe burn, BNSB: burned area of non–severe burn NBNSB: non–burned area of non–severe burn.

Immunohistochemistry for VE–cadherin. The samples of severe burned group showing the distribution of fine brown granules of VE–cadherin in veins as follow. BSB group shows a low intensity granules distribution in both of attached and detached endothelium and NBSB group shows a high intensity granules distribution in both of attached and detached endothelium. The samples of non–severe burn group show a low intensity in both of BNSB– and NBNSB group. In the capillaries, BSB group show a low intensity granules distribution in both of attached and detached endothelium. NBSB group shows a high intensity granules distribution in both of attached and detached endothelium. The samples of non–severe burn group showing the distribution as follow. NBSB group shows a high intensity granules distribution. NBNSB group shows a low intensity granules distribution (fig.2).

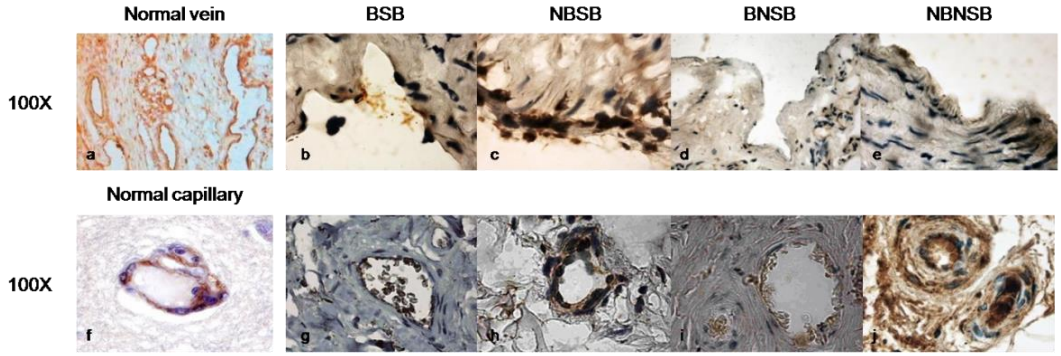

Figure 2. The immunohistochemistry of vein and capillaries in burned injured samples using VE–cadherin antibody in objective lens magnification of 100 times. a. A normal vein of a young healthy volunteer showing the distribution of fine brown granules of VE–cadherin in strong intensity around the endothelial cells. b. Vein of BSB showing the distribution of fine brown granules of VE–cadherin in strong intensity around endothelial cells attached to the basal membrane of tunica intima. c. Vein of NBSB showing the distribution of fine brown granules of VE–cadherin in strong intensity around both attached and detached endothelial cells. d. Vein of BNSB showing the distribution of fine brown granules a weak intensity around detached endothelial cells. e. Vein of NBNSB showing Vein of BNSB distribution of fine brown granules a weak intensity around detached endothelial cells. f. Normal capillary showing the distribution of fine brown granules of VE–cadherin in strong intensity around the endothelial cells. g. Capillary of BSB showing the distribution of fine brown granules in a weak intensity around detached endothelial cells. h. Capillary of NBSB showing the distribution of fine brown granules a weak intensity around attached endothelial cells. i. Capillary of BNSB showing the distribution of fine brown granules a weak intensity around detached endothelial cells. j. Capillary of NBNSB showing the distribution of fine brown granules in strong intensity around attached endothelial cells. BSB: burned area of severe burn, NBSB: non–burned area of severe burn, BNSB: burned area of non–severe burn NBNSB: non–burned area of non–severe burn.

Table 2. Statistical analysis of fine brown granules of VE–cadherin in the samples of vein and capillary

	Group	Mean ± SD		p (CI 95%)
		B (n = 10)	NB (n = 4)	
Vein	BSB	3.200 ± 0.632	3.666 ± 0.577	0.279 (–1.369–0.435)
	BNSB	3.000 ± 0.866	3.000 ± 1.154	0.303 (–1.595–0.662)
Capillary	BSB	2.666 ± 0.816	2.125 ± 0.640	0.553 (–0.930–1.597)
	BNSB	2.333 ± 0.577	2.00 ± 0.816	0.776 (–0.827–1.077)

In the analysis, the most score of granules distribution in the veins of burned area found is 4 (60%), whereas in veins of non–burned area is 3 (48%). While as the most common found score of granules distribution in the capillaries of burned area is 2 (36%) and of non–burned area is 2 (52%)(table 2).

Sensitivity and specificity test showed a low value in veins of severe – and non – severe burn, both in burned– (0.259) and non–burned area (0.38), and so was the capillaries both of burned– (0.667) and non–burned area (0.533).

Immunohistochemistry for occludin. The samples of moderate size veins of severe burned group showing the distribution of brown granules of occludin in veins as follow. BSB group show a low intensity distribution of the granules in both of attached and detached endothelium. NBSB show a high intensity distribution of the granules. The samples of moderate size veins of non–severe burn group, the samples of both of BNSB and NBNSB group are of low intensity distribution of the granules (table 3).

In the analysis, the most common found score of granules distribution in the veins of burned area is 3 (53.8%), whereas in veins of non–burned area is 2 and 4 (each of 38.5%). Whilst most common found score of granules distribution in the capillaries of burned area is 2 (55.6%) and of non–burned area is 2 (58.3%).

Sensitivity and specificity test showed a low value in veins of severe– and non–severe burn, both in burned– (0.486) and non–burned area (0.583), and so was the capillaries both of burned– (0.500) and non–burned area (0.607).

The expressions of mRNA of VE–cadherin and occludin were carried out through the calculation of cycle threshold (Ct) semi–quantitatively using Real Time–PCR. There were eighteen samples preceded the test which is carried out simultaneously in duplex. Mean of Ct is used to calculate both expressions using equation according to the method of Livak. Such an mRNA expression is expressed in ratio to beta–actin as housekeeping gene; and the expression mRNA of burned area is to be compared to non–burned area whilst severe– is to be compared to non–severe burn. The cycle threshold (Ct) of mRNA of VE–cadherin of burned area is found earlier (26th cycles), before the Ct of mRNA of beta–actin (29th cycles). Whereas the Ct of mRNA of occludin of burned area is found later, after the Ct of mRNA of beta–actin (33th cycles). Furthermore, through the calculation of expression ratio, it is found that the expression of mRNA of VE–cadherin to be increased (4.397) and expression of

mRNA of occludin to be decreased (0.040). In severe burn group, the Ct of mRNA of VE–cadherin is found to be detected earlier (29th cycles) before the Ct of mRNA of beta–actin (32th cycles) and also the Ct of mRNA of occludin (31st cycles). Further calculation of expression ratio shows that the expression of mRNA of VE–cadherin (1.747) is to be increased as well as the expression of mRNA of occludin (1.887).

Table 3 Incidence of catheter–related bacteremia

Variable	Infection		No Infection		p	*OR (CI95%)*
Type of DLC	n	%	n	%		
• Non–tunneled	14	26.4	39	73.6	0.043	3.446 (1.141–10.406)
• Tunneled	5	9.4	48	90.6	1.00	
Total incidence of DLC	19	17.9	87	82.1		

Table 4. Factors related to bacteremia after double lumen catheter insertion

Variable	Infection		No Infection		P	*OR (CI95%)*
Type of double–lumen catheter	n	%	n	%		
• Non–tunneled						
• Tunneled	14	26.4	39	73.6	0.043	3.446 (1.141–10.406)
Gender	5	9.4	48	90.6	1.00	
• Male	14	24.6	43	75.4	0.095	2.865 (0.950–8.644)
• Female	5	10.2	44	89.8	1.00	
Age						
• >60 years old	7	30.4	16	69.6	0.076	2.589 (0.880–7.611)
• 18–60 years old	12	14.5	71	85.5	1.00	
Diabetes Mellitus						
• Yes	8	26.7	22	73.3	0.233	2.149 (0.766–6.025)
• No	11	14.5	65	85.5	1.00	
Hypertension						
• Yes	11	19.6	45	80.4	0.815	1.283 (0.471–3.499)
• No	8	16.0	42	84.0	1.00	
Body Mass Index						
• <18.5	2	14.3	12	85.7	0.552	0.735 (0.150–3.594)
• 18.5– 25 and >25	17	18.5	75	81.5	1.00	
History of previous catheter insertion for hemodialysis						
• Yes	9	12.2	65	87.8	1.00	
• No	10	31.3	22	68.8	0.038	0.305 (0.110–0.847)
History of previous bloodstream infections						
• Yes	4	19	17	81	0.550	1.098 (0.323–3.733)
• No	15	17.6	70	82.4	1.00	
Site of insertion						
• Femoral	12	23.5	39	76.5	0.232	2.110 (0.758–5.871)
• Jugular and Sub clavicular	7	12.7	48	87.3	1.00	
Duration of use						
• >2 weeks	16	18.8	69	81.2	0.450	1.391 (0.365–5.302)
• ≤2 weeks	3	14.3	18	85.7	1.00	

Discussion

The endothelial cell–to–cell junction is first described by Dejana and colleagues in 1995.[14] At the later date, it is known that endothelium have similarity to epithelium, which has three cell–to–cell junctions, i.e. tight junction, adherens junction and gap junction.[14] During the process of inflammation, it was believed that the adherens junction is the only junction prone to be dissociated following an exposure to inflammatory mediators, and such a change is in found temporarily in reversible manner.[14,15] More than 10 years later, tight junction which is never thought to be involved eventually is also having a tendency as adherens junction is, even though in a well–protected blood brain barrier.[16]

With increased of capillary permeability during the process of inflammation which is endothelial cell–to–cell junction disassembly eventually, there are dissociations of assembled junction adhesive molecules; occludin of tight junction and VE–cadherin of adherens junction.[14,15,16] There are also might be dissociation of beta–actin of cytoskeleton and integrin of endothelial cell–to–matrix dissociation.[17]

Normally, the distribution and intensity of the brown granules of VE–cadherin is found to be finest and less intense than occludin. This is due to dissociation of VE–cadherin is running out faster than the dissociation of occludin. It is clearly described in the earlier literature (1986–1997) and then reviewed 20 years later (2007–2011) that VE–cadherin is undetectable in minutes to hours following a trauma. Yet VE–cadherin is the first molecules comes to be detectable as soon as the circulation is recovered following shock.[19] However occludin dissociates slower than VE–cadherin and could be detected in 6 hours as the circulation is recovered following the exposure to various stimuli (such as inflammatory mediators, hypoxia, etc.).

The tight junction which is in the most apical side considerably referred to the first junction to be exposed to a stimulus, though to be dissociated lately. This explains why the intensity of occludin is to be found different and more intense than VE–cadherin in the immunohistochemistry exam. Somehow, there is no data about this information in burns has ever been published. The samples of vessels obtained in the period of >8–72 hours post burn show both

endothelial cell–to–cell junction molecules, namely VE–cadherin and occludin in term of decreasing of the distribution and intensities as well. The evidence of any dissociation (degradation) of molecules it is realized to be ascertained by furthers biochemical study.

However, in severe burn the score that representing distribution and intensity of VE–cadherin in the vessels of burned area is found not to be different significantly to non–burned area (p 0.279). It is also found in the vessels of non–severe burn that burned area is found not to be different significantly to non–burned area (p 0.303). The vessels of burned area showed that the most common score of distribution and intensity of VE–cadherin found is 4 (50%), whereas of non–burn area are 2 and 3 (each of 35.7%). Whilst in the capillaries, the most common score found is 2 (36%) and non–burned area is 2 (52%). Then, the sensitivity and specificity test (in burn area of 0.259 and non–burn area of 0.389) showed that these values were not representing the characteristic of severe burn.

The scores representing distribution and intensity of occludin in the vessels of burned area shows no significant difference statistically to non–burned area (p = 0.913). We also found similarity in non–severe burn, that burned area shows no significant difference statistically to non–burned area (p = 0.893). As seen in VE–cadherin, the frequent score found in distribution and intensity of occludin did not representing the characteristic of severe burn as sensitivity and specificity test shows of low values (burned area 0.486 and non–burned area 0.583). Again, it was found similar in non–severe burn (burned area of 0.514 and non–burned area of 0.417).

Increased expression of mRNA reveals the information of ongoing synthesis following dissociation (degradation) of molecules which is shown by the early study of immunohistochemistry. In contrast, any decrease of the expression represents whether there is no synthesis or delayed one. We found that in group of burned area, the expression of mRNA of VE–cadherin is increased whereas the expression of mRNA of occludin is decreased. Somehow, in group of severe burn we found the expression of mRNA both of VE–cadherin and occludin are increased.

These increased syntheses both of mRNA of VE–cadherin and occludin indicate ongoing its dissociation (or – the worst – degradation) of these junctional molecules. The synthesis is meant to replace molecules which are dissociated, in context of homeostasis. In other perspective, such an increase of expression which is observed within >8–72 hours indicate there's no cellular injury found yet (no apoptosis, neither necrosis). This was found paralleled to the value of mixed vein oxygen saturation (SvO2) in our previous study revealing the process of adaptive phase following injury.

Finally this study showed that even our former study of histomorphology of endothelial lining as well as study of immunohistochemistry showed no significant difference statistically between severe and non–severe burn,[13] but our study in clinical aspect and molecular study of views representing the synthesis of mRNA of VE–cadherin in burned area, increased both synthesis of mRNA of VE–cadherin and occludin in non–burned area indicates the dissociation of adhesive endothelial cell–to–cell junctional molecules.

There are several influencing factors were thought to be responsible for this endothelial cell–to–cell junction dissociation, namely inflammation, hypoxia, oxidative stress, ischemia–reperfusion injury, cell injury, hyperglycemia and toxic effect of lipid protein complex (LPC).

Inflammation. The process of inflammation as the body response to injury led to endothelial changes namely endothelial junction (both of endothelial cell–to–cell junction and endothelial cell–to–extracellular matrix junction) disassembly. There are known factors affecting this kind of changes, i.e. leukocytes and inflammatory mediators such as tumor necrosis factor (TNF□) and interleukin (IL).20,21 In both of groups, the inflammatory response is represented by the increased of leukocyte count, high level of both of C–reactive protein (CRP) and Procalcitonin (PCT) and prolonged partial thromboplastin time, which has been showed in our previous study.22

Endothelial changes in inflammation. Dissociation of endothelial junction is not of standalone factor but found together with morphological changes. Though is not clear what was initiating the other, the fact is both changes were clearly identified. The endothelial morphological changes are showed under microscopic examination using hematoxylin eosin staining,13,23 and molecular exams showed by alteration of beta–actin expression representing the dissociation of cytoskeleton. Endothelium morphological changes which is found together with junction molecules dissociation revealing that endothelial barrier is no longer maintained, in other word there is endothelial dysfunction.24

Hypoxia. In both of group subjected to such investigation, the increased of PCO2 were objectively identified, though this was not the main subject of last discussion. The established standard exam used to find out the evidence of hypoxia is by measuring hypoxia inducible factor (HIF–1□). There was evidence of HIF–1□ showed a correlation with junction molecules dissociation.25 Unfortunately HIF–1□ is not designed to be the object in our study.

Oxidative stress and lipid peroxidation. The information is provided by the level of serum malondialdehyde (MDA), which is increased in acutely injured burn patients. Alfara who used the same sample in this investigation focused on the efficacy of vitamin C and vitamin E administration, shown that serum MDA (normal 0.062–0.118 nmol/L) drastically increased in severe burn (ranged of 1.078–4.379, mean of 2.096, in non–severe burn ranged of 0.446–0.941, mean of 0.748 nmol/L);26 which if similar to those reported by Nielsen in his findings on burn (0.72–4.38 nmol/L),27 and was higher a little bit than works reported by Horton.28 This was found higher a lot compared to healthy peoples having oxidative stress (0.157 nmol/L). The works of Alfara provides valuable information of systemic stress oxidation since serum MDA was the parameter being observed and somehow it provides no clear information of what specific cells was being stressed. By all means, burn shock led to lack of perfusion and cellular oxygenation and followed by oxidative stress. And endothelium is not exclusively relieved from such a condition. Hence, there should be a further study to find out the evidence of oxidative stress in the cellular level, particularly endothelium in burn.26

Ischemia–reperfusion injury. To find out the evidence of reperfusion injury one should carry out some tests measuring xanthine–oxidase. This kind of test is not yet being exam to endothelium as there insufficient information.29

Cells injury. Serum lactate dehydrogenase (LDH) is now established as a standard procedure to find out the evidence of cells injury. Consideration of specific test for endothelium is to be advised.30

Hyperglycemia. Studies showed that hyperglycemia in acute burned injury led to protein catabolism, endothelial changes and junction dissociation. Hypoxic membrane is followed by inefficacy of insulin receptor which is a trans–membrane protein and the integral protein of a membrane.32 Thus the physiologic process of glucose diffusion

across the membrane is somehow disrupted; leading to hyperglycemia despite stressed mitochondria though studies in burns remain scanty. In previous study, acute hyperglycemia was identified in both of severe– and non–severe burn group.22, 30,32,33,34
Lipid protein complex (LPC) formerly known as burn toxin35 which is through a long way to run proven as pernicious effector in burns36 to be toxic to hepatocyte (apoptosis), and erythrocytes (echinocytes) as it led to cells lipoprotein polymerization. There's insufficiency data consider its effect to endothelium.

In some way not yet known or designated in this study of immunohistochemistry and the expression of mRNA of VE–cadherin and occludin enfaced limitations. One of limitation the number of samples that might be affecting the outcome. Such problems encountered are patient and sample enrollment. There were patients who were not willing to join in regarding the procedure of biopsy to take the samples of non–burned area, especially in non–severe burn. This was considered earlier as this kind of investigation is performed in human.

Conclusion

The molecular study showed that instead of dissociation of VE–Cadherin there were dissociation of occludin with no differences between the two groups, and between burned– and non–burned areas as well.

Conflict of interest

Author disclose no conflict of interest.

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