A Simple Method to Measure Serum Lactate Concentration as A Reliable Parameter to Detect Flaps Blood-flow Patency

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Background: The change in flap blood flow patency can be monitored by measures. Subjective clinical observation through flap examinations is greatly biased depending on examiner's experience. Standardised equipments of assessment are more reliable. This study propose the use of a device to provide an objective, safe, reproducible, simple, portable, and cost-effective method of monitoring flaps vitality by measuring serum concentration of lactic acid.
Methods: An experimental lab-based study was conducted utilising Sprague-Dawley rats. Twenty rats were randomly assigned into two groups. In all subjects, bilateral groin-flaps were elevated. In rats of Group A, the vein pedicle of one-side of the flap was occluded while in Group B both the artery and vein on one-side of the flap were occluded. The other side of flaps in each rat were left unoccluded to serve as controls. Baseline serum lactate was measured in all flaps, then remeasured 60 and 120 minutes in all flaps after pedicle manipulations.
Results: The mean lactate concentration of Group A rats with vein-occluded flaps was 2.5 ± 0.17 mmol/L at 0-min initially, and increased to 7.9 ± 0.16 mmol/L 120-min after occlusion ($p<0.0005$). The mean lactate concentration of Group B rats with arterial and venous flap occlusion was 2.55 ± 0.21 at baseline, which increased four-fold at 120-min to 9.86 ± 0.28 mmol/L ($p<0.0005$). Among the two groups, the lactate difference was also found to be significant.
Conclusion: This study demonstrates that the proposed method detects serum lactate changes in flaps with vein and arteriovenous occlusions. This thus can be used as an objective parameter to evaluate compromised blood flow on cutaneous flaps.
Keywords: *Flap monitoring, serum lactate, portable flap monitoring*
Latar Belakang: Perubahan patensi aliran darah yang memperdarahi suatu flap dapat dipantau menggunakan beberapa metode. Pemantauan klinis secara subjektif melalui temuan fisik yang sering dilakukan sangatlah bias dan bergantung pengalaman observer. Peralatan terstandardisasi untuk monitoring lebih dapat dipercaya. Studi ini mengajukan penggunaan suatu metode objektif untuk monitoring flap secara objektif, aman, sederhana, portabel, dan ekonomis; dengan mengukur kadar asam laktat dalam darah.
Metode: Studi experimental laboratoris dilakukan menggunakan tikus Sprague-Dawley. Dua puluh tikus dirandomisasi dalam dua group intervensi. Flap inguinal bilateral dielevasi pada tiap tikus. Pada Grup A, vena dari pedikel di salah satu sisi flap dioklusi dan pada tikus Grup B arteri dan vena dari pedikel pada salah satu flap dioklusi. Salah satu sisi flap tidak dioklusi sebagai kontrol. Kadar laktat pada semua flap diukur sebelum intervensi, dan 60 serta 120 menit setelah pedikel dimanipulasi.
Hasil: Nilai rata-rata kadar laktat di Grup A pada flap yang venanya dioklusi 2.5 ± 0.17 mmol/L pada awal, dan naik menjadi 7.9 ± 0.16 mmol/L 120 menit setelah oklusi ($p<0.0005$), sementara pada flap Grup B yang arteri dan venanya dioklusi kadar laktat awal 2.55 ± 0.21 naik empat kali lipat menjadi .86 ± 0.28 mmol/L ($p<0.0005$) 2 jam setelah oklusi. Kadar laktat antara kedua grup juga berbeda secara signifikan.
Kesimpulan: Studi ini menunjukkan bahwa metode yang digunakan dapat mendeteksi perubahan kadar laktat dalam darah pada flap yang dioklusikan vena dan arteriovenanya. Dengan demikian metode ini dapat digunakan sebagai parameter objektif untuk mengevaluasi perubahan aliran darah pada flap kutan.
Kata Kunci: *Flap monitoring, serum lactate, portable flap monitoring*

The reconstruction of defects from tissue loss or damage has favored the use of microsurgical free-tissue transfer technique in the past three decades. The advancement of techniques in microsurgery and the availability of more skilled microsurgeons contribute to the higher success rate of free-tissue transfer nowadays. However,

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Presented in The 14th IAPS Scientific Meetings In Balikpapan, West Kalimantan

Disclosure: *The authors have no financial interest to declare or commercial affiliations in relation to the content of this article.*

1 to 10% of free flaps still fail. To prevent this, any suspicion of vascular disturbance to a flap should be detected early as this may salvage the flap.[1-3]

When the vascularity of a flap is compromised hypoxia will ensue, shifting the metabolism from aerobic into anaerobic thus resulting in pyruvate a build-up which are then converted into lactic acid. Lactic acid concentration can be detected in a small volume of blood (15-20 uL/L) via a portable device commonly used in the Intensive Care Unit.[5,6] In the conventional monitoring, pinprick test is evaluated by the color of the blood drop, and the rate of its emergence. The small amount of blood acquired from the pinprick test can be assessed using a portable device to detect its lactate concentration, which reflects the local lactate content within an ischemic flap. In our intensive care unit, Ade and Michael (2008, unpublished) utilized a portable lactate analyzer to measure serum lactate concentration among their patients to detect a state of tissue hypoperfusion as a predictor of mortality.

The proposed technique in this study evaluates flap patency by utilizing a device to measure serum lactic acid level which is quick in producing interpretation, objective, safe, reusable, simple, portable, and cost-effective.[7] By means of the conventional pinprick test, a drop of blood sample is drawn from the flaps and tested using the device. This may be implemented as a reliable parameter to assess the vitality of flaps and detect compromised vascularity.

METHODS

An experimental lab-based study was conducted in the Central Laboratory of Research and Development for Diseases Eradication, Division of Research and Development for Health, Department of Health, at Jalan Salemba Raya 6 Central Jakarta on June 2009 utilizing pure locally-bred Sprague-Dawley rats. Animal ethical clearance was obtained.

Healthy adolescent male rats 3 to 4 month-old which weighed between 300-400 gram were included in the study. All rats received the same type of food, 30-40 g pellets per day composed of: 25% protein, 12% fat, 53% carbohydrate, 4% fibers, 2% calcium phosphate, and 5% ash. Subjects are eliminated from the study when the rats became unwell, indicated by reduced activeness, weakened appearance, and food-intake refusal.

A total of 20 rats were randomly assigned into two groups of intervention. In all subjects, a 1 by 2 cm bilateral groin-flap were elevated microsurgically. In rats of Group A, the vein pedicle of one-side of the flap was occluded while in Group B both the artery and vein on one-side of the flap were occluded. The other side of groin flaps in each rat were left unoccluded and serve as controls. Baseline serum lactate was measured in both flaps of all subjects, flaps were sutured back into place and then lactate serum level remeasured 60 and 120 minutes in all flaps after pedicle manipulations. Blood samples were obtained by pinprick from the flap edges (15-50 ul/L), dripped onto a BM Lactate Stick® then analyzed on a portable analyzer device (Accutrend®).

After randomized group allocation, rats were knocked down using intramuscular injection of combined 50 mg/kg body weight Ketamine and 5 mg/kg Xylazine. Depth of anaesthesia were monitored by stroking the rats whiskers and pinching of intertoe-webs. Wiggling of the whiskers in response to touch, and reaction to web-pinching indicated an awakening and a maintenance intramuscular Ketamine injection (5 mg/kg) was readministered. Hair around the groin areas was trimmed prior to commencement of surgery. Groin-flap design were drawn on both sides of rats groin, flaps elevated micro-surgically, and vascular pedicles preserved (Figure 1).

In rats allocated to Group A, the vein pedicle of one groin-flap side is ligated (Figure 2 left), the contralateral side left unligated as a control. Rats within Group B underwent the same procedures, but with the artery and vein on one side of the groin-flap ligated (Figure 2 right), the contralateral side left as a control. Baseline serum lactate level was obtained by pinprick before ligations. After ligations, flaps

Figure 1. Groin-flap design (left), elevation (middle), and vascular pedicle preservation (right)

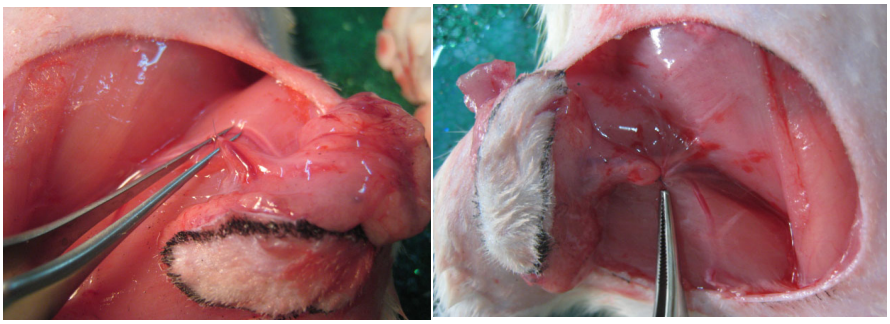

Figure 2. After full flap elevation, vein in Group A rats were occluded (left), and of the artery and vein of Group B rats were occluded (right).

were insetted back into place and blood samples obtained 60 then 120 minutes after vascular occlusions. At the end of flaps observation, pedicles were ligated, flaps removed, and wound sutured primarily. Rats were finally euthanized by administering a lethal dose of Ketamine.

RESULT

Data obtained was analyzed using the paired student t-test. All 20 rat subjects survived until the end of study and are included in the analysis. The mean serum lactate of Group A rats with vein-occluded pedicle was 2.5 ± 0.17 mmol/L at 0-min prior to occlusion, and increased to 7.9 ± 0.16 mmol/L 120-min after occlusion (Figure 3). T-test revealed p-value <0.0005. The mean lactate concentration of Group B rats with arterial and venous flap occlusion was 2.55 ± 0.21 at baseline, which increased more than four-fold at 120-min to 9.86 ± 0.28 ($p < 0.0005$, Figure 4). The difference in serum lactate between the two groups at 120-min was found to be significant at $p < 0.0005$. Lactate concentrations in the control group remained unchanged throughout (Figure 5).

DISCUSSION

Monitoring the vitality of flaps is essential to assure the success of a microsurgical free-flap. Early identification of vascular compromise validate the need for an immediate intervention to improve circulation thus salvaging the flap. Numerous methods of monitoring can be done, the ideal being one which is quick in interpreting results, objective, safe, easy to apply clinically, reproducible, portable, and affordable.

This study aim to determine whether the increased lactate level detected locally on a circulatory-deprived flap can be used as an objective parameter to evaluate a state of vascular compromise. A drop of blood sample was obtained by pin-prick test from the flap edges and then examined using a portable lactate analyzer, a device commonly used in the Intensive Care Unit, with a mechanism similar to that of diabetic's gluco-stick test using a portable glucose analyzer.

The results demonstrate that an increase in local serum lactate concentration justify a state of vascular insufficience in a

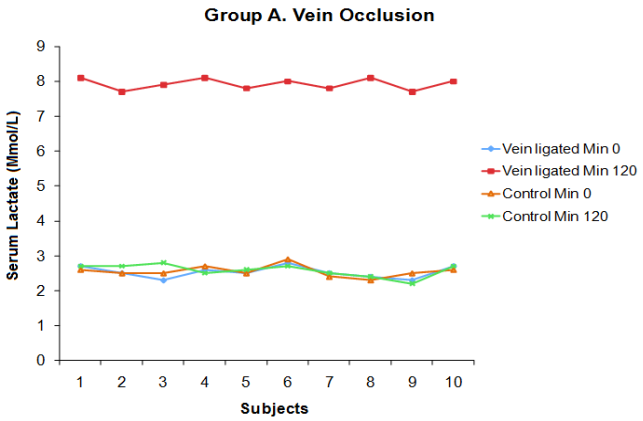

Figure 3. Serum lactate change in vein-occulded flaps before ligation and 120 minutes after ligation, compared to controls. In unligated flaps as well as controls, serum lactate remained constant.

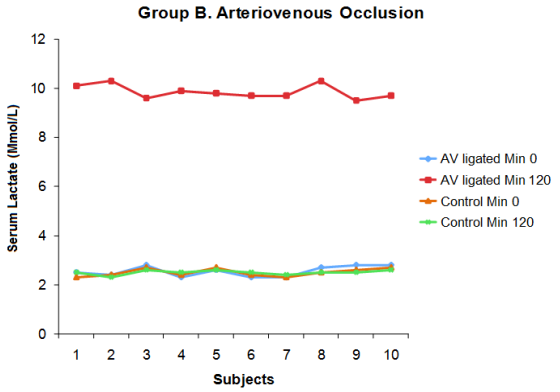

Figure 4. Serum lactate change in arteriovenous-occulded flaps before ligation and 120 minutes after ligation, compared to controls. Lactate concentration is higher in this group than the vein-occluded group at 120-min.

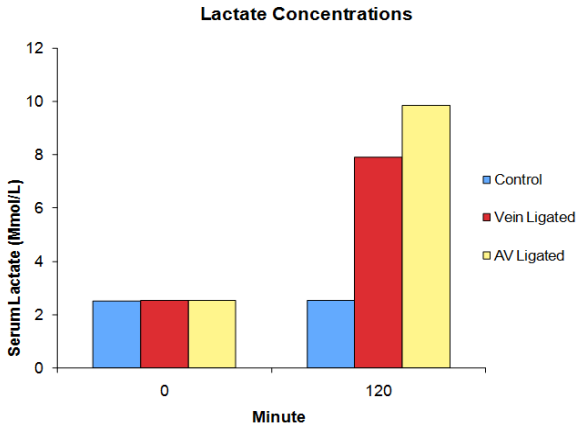

Figure 5. Serum lactate change in controls, vein-occluded rats, and arteriovenous-occluded rats: before pedicle manipulation and 120-min after occlusion.

flap. In the vein-occluded flaps, lactate increased three-fold whilst in the arteriovenous-occluded flaps the lactate increased almost four-folds 2 hour postocclusion. Statistical analysis compares the lactate of: vein occlusion versus control, arteriovenous occlusion versus control, vein occlusion versus arteriovenous occlusion; 120 minutes after manipulation. Differences were found among each comparison and were statistically significant. The measured result reflects a local serum lactate level of corresponding local ischemic tissues and does not reflect a systemic lactate concentration.

Setala and colleagues evaluated the metabolic changes within flaps with compromised blood flow using microdialysis with catalyzed spectrophotometric assay and measurement of the kinetic enzymatic reaction.[7] One of the measured metabolytes was lactate, and they found that the serum lactate concentration of vein-occluded flaps increased 3.6 times 2-hour after ligation, and increased 6.7 times higher in the artery-occluded flaps. Although the results are significant and microdialysis is proved to be sensitive in detecting lactate level, the procedure itself is fairly invasive with the need of inserting microdialisate tubes between flap sutures, complicated by occasional tube obstruction. In 1982, Tsung also evaluated the metabolic serum lactate changes in flaps with compromised

Serum lactate were found to be elevated in vein-occluded as well as arteriovenous-occluded flaps (3.5-4.5 times and 6 times higher respectively) within four hours after pedicle manipulation.

Serum lactate level builds up gradually when venous and/or arterial blood flow decreases because the tissue is hypoxic. Lactate level increases higher when arterial circulation is occluded compared to vein. Partial aerobic metabolism from glycolysis takes place when vein is compromised because the artery still carries the oxygen. The findings from this animal study is expected to be applied on humans. Findings from the conventional flap monitoring by subjective clinical observation can be supported and further reinforced by the objective serum lactate measurement. This method is especially helpful for muscle flaps without exteriorized skin component which can be clinically observed. Slight elevation of serum lactate indicates an early circulatory compromise, hence the necessary salvage attempts can be initiated. The use of Doppler ultrasound for monitoring such muscle flaps pose the difficulty of differentiating the local tissue vascular flow with the main arterial and venous pedicles which lie near it.[8]

CONCLUSION

Monitoring lactate level using this device can be done quickly (within 60 seconds), and does not require an extra purchase because the portable lactate analyzer can mostly be found in the intensive care unit of hospitals. It allows a quick, simple, portable, reproducible, and cost-effective metod of evaluating flaps.

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