The difference in Abdomen and Anus

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The Difference in Abdomen and Anus Temperature in Mice with Various Conditions

(PERBEDAAN SUHU ABDOMEN DAN ANUS PADA MENCIT DENGAN BERBAGAI KONDISI)

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ABSTRACT

Research about sepsis in animal model is interesting to get the right therapeutic method for human being. We did the research to study about the value of body temperature in mice's various body area in order to get the important information if we are going to do the translational research in animal model about sepsis. One clinical signs of the sepsis mice model is changes in body temperature. An easy way to examine body temperature is using infrared non-contact thermometer. This study aimed to compare the body temperature using infrared non-contact thermometer at the abdomen and anal area. We used male mice, weighing 25–30 g, divided into two groups (control and treatment groups). The control group injected with NaCl 0.9% solution, with the amount of NaCl 0.9% volume equal to Lipopolysaccharide (LPS). In the treatment group injected with 2.5 mg/kg BW of LPS intraperitoneally. Body temperature was measured in abdomen (t_{abd}) and ananl (t_{an}) area at 8th and 24th hour after treatment. Body temperature value t_{abd} was higher than t_{an}. Lipopolysaccharide injection increase body temperature but was not significant when compared to the control group (8th and 24th hour). The mean difference between t_{abd} and t_{an} in 8th control groups were 2.12^{o}C respectively. The mean difference between t_{abd} and t_{an} in 24th hour control groups 4.6^{o}C. The mean difference in treatment groups (8th hour) was 4.66^{o}C, while it was 4.77^{o}C in the 24th groups. Giving 2.5 mg/kgBW LPS intraperitoneally did not rise the body temperature significantly as compare to control groups. But, body temperature at anus area using non-contact infrared thermometer after treatment showed lower results as compared to that of at abdomen significantly.

Keywords: anus temperature; abdomen temperature; non-contact infrared thermometer; non-invasive thermometer

ABSTRAK

Penelitian tentang sepsis pada model hewan sangat menarik untuk mendapatkan metode terapi yang tepat bagi manusia. Kami melakukan penelitian untuk mempelajari nilai suhu tubuh pada berbagai area tubuh mencit untuk mendapatkan informasi penting jika akan melakukan penelitian translasi pada model hewan tentang sepsis. Salah satu tanda klinis model mencit sepsis adalah perubahan suhu tubuh.

Cara mudah untuk memeriksa suhu tubuh adalah dengan menggunakan termometer non-kontak inframerah. Penelitian ini bertujuan untuk membandingkan suhu tubuh dengan menggunakan termometer non kontak infra merah pada daerah abdomen dan anus. Kami menggunakan mencit jantan dengan bobot 25–30 g, dibagi menjadi dua kelompok (kelompok kontrol dan perlakuan). Kelompok kontrol diinjeksi dengan larutan NaCl 0,9%, dengan jumlah volume NaCl 0,9% sama dengan lipopolisakarida (LPS). Pada kelompok perlakuan diinjeksi dengan LPS 2,5 mg/kg BB secara intraperitoneal. Suhu tubuh diukur di daerah abdomen (t_{abd}) dan anus (t_{an}) pada jam ke-8 dan 24 setelah perlakuan. Nilai suhu tubuh pada abdomen (t_{abd}) lebih tinggi dari suhu pada anus (t_{an}). Injeksi LPS meningkatkan suhu tubuh tetapi tidak signifikan jika dibandingkan dengan kelompok kontrol (jam ke-8 dan ke-24). Perbedaan rata-rata antara t_{abd} dan t_{an} pada jam ke-8 kelompok kontrol, masing-masing adalah 2,12 fC. Perbedaan rata-rata antara t_{abd} dan t_{an} pada kelompok kontrol setelah 24 jam injeksi 4,6°C. Perbedaan rata-rata pada kelompok perlakuan (jam ke-8) adalah 4,66 °C, sedangkan pada jam ke-24 adalah 4,77 °C. Pemberian LPS 2,5 mg/kg BB secara intraperitoneal tidak meningkatkan suhu tubuh secara signifikan dibandingkan dengan kelompok kontrol. Namun, suhu tubuh di daerah anus dengan menggunakan termometer infra merah non-kontak setelah perlakuan secara nyata menunjukkan hasil yang lebih rendah dibandingkan suhu di abdomen.

Kata-kata kunci: suhu anus; suhu abdomen; termometer inframerah non-kontak; termometer non-invasif

INTRODUCTION

Temperature measurement is an important indicator of infection in laboratory animal models. We can measure body temperature in the anus or abdomen. The commonly used method is by a thermometer in which the tip is inserted into the anus. Today, infrared thermometers are often used in humans to measure body temperature. One of the advantages of using this thermometer is to avoid the spread of infection because the device does not touch the skin. We tested the comparison of the value of body temperature measurement in anus and abdomen area using non-contact infra red thermometer in male mice. Non-contact infrared thermometer has time efficiency (~3-4 seconds per measurement), high experimenter-dependency, and high accuracy to predict death as an outcome even (Mei *et al*., 2018). Besides measuring the difference in temperature measurement values in the anus and abdominal area, we also measured for an increase in body temperature in the control group and treatment after giving LPS 2.5 mg/kg intraperitoneally as one of sepsis signs .

Temperature measurement in mice is crucial in investigating body's physiological changes due to infection and temperature is still used as a sign of infection. Rectal thermometer is the most commonly used device due to accuracy concern. However, this technique of measurement has several weaknesses including: requirement of anesthesia in mice to avoid injury (Kawakami *et al*., 2018), the device is relatively expensive, and repeated measurements may cause death in mice (Suckow *et al*., 2001). Mice included in the order of rodentia, family muridae (Lundrigan *et al*., 2002). These mice have genetic and biological characteristics of mammals. They were first used in the 17th century for respiration study. At the end of the 19th and 20th centuries, these mice were farmed according to laboratory requirements based on skin color and genetic characteristics. Laboratory strains were developed in the early 1900s (Whary *et al*., 2015). Room conditions for mice are generally maintained at relative humidity of 30-70% and room temperature of 18-26°C. The recommendations written by Speakman and Keijer (2013) stated that room temperature for mice was 20-26°C ("National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals," 2011), 20-24°C (Havenaar *et al*., 2001) or 19-23°C. The room temperature in laboratory is generally set to 20-22°C, to also ensure the comfortability of the staffs (Speakman and Keijer, 2013).

The body temperatures of laboratory mice are commonly measured with rectal thermometers. This methode may be an uncomfortable and stressfull procedure, and repeated insertion of rectal temperature probes can cause mucosal tears, leading to septicemia and death (Saegusa and Tabata, 2003). The body temperature of mice varies depending on the strain and measurement method, but the average temperature of mice is 36.5-38.0°C (Suckow *et al*., 2001).

RESEARCH METHODS

This research has been approved ethical permission from the Ethical Committee of Faculty of Medicine, Universitas Airlangga, with the ethical conduct number: 2.KE.063.04.2019.

Experimental Animals

Inclusion criteria includes: male *Mus musculus* mice, 12-16 weeks old, weighing of 25-30 g, obtained from Center for Laboratory Experimental Animals, Universitas Gadjah Mada. The mice adapted for a week, with adequate food (normal diet given in pellets form consist of 70% carbohydrates, 20% protein and 10% fat made in laboratory) and lighting. Exclusion criteria includes: mice's general condition seemed weak before experiment and mice which appeared aggressive. Drop out criteria includes death of the mice during trial period.

Experimental Design

Lipopolysacharide (LPS) (InvivoGen LPS-EB (LEB-38-02)) was obtained from *Escherichia coli*. The LPS was diluted with normal saline to contain 0.025 mg for every 0.1 mL of liquid. Treated group was given LPS with the dose of 2.5 mg/kg BW, injected intraperitoneally. Control group was given intraperitoneal NaCl 0.9% injection with equal volume as LPS.

Each groups consisted of 10 mice. Mice in both treatment and control groups were further divided into two groups: 8th hour after LPS or NaCl 0.9% injection and 24th hour after LPS or NaCl 0.9% injection groups.

Body Temperature Measurement

We measured mice body temperature using thermometer (Thermoworks TW2®, Lindon, USA). The measurement were at abdomen (t_{abd}) and anus (t_{an}) regions. Room temperature was according to air conditioner temperature, which was 16°C. Body temperature was measured at 8th and 24th hour after intraperitoneal injection of LPS or NaCl 0.9% and the results were then compared.

Statistical Analysis.

We analized the value of the results in the two groups using SPSS for Mac 21.00. The value of body measurement in abdomen area were significantly different with the value in the anus area, both in the control and treatment groups (Table 4). This information is important for the researcher to choose the best technique for measuring body temperature in mice.

RESULTS AND DISCUSSION

Mice injected with LPS showed decreased activity at the first hour post-LPS-injection. They further deteriorated at 8th hour post-injection and appeared weakest at 24th hour post-injection. Mice in control group did not show any changes in activity at 1st, 8th, or 24th hour post-NaCl 0.9%-injection.

The body temperature measured (Figure 1) at abdomen and anus regions 8th and 24th hour post-injection are presented in Table 1 and 2. Table 1 shows the result of the body temperature measurement (LPS groups) and Table 2 shows the result of the body temperature measurement (NaCl 0.9% groups). Those groups devided into 8th hour group and 24th groups measurement. Each group consist of 10 male mice. In the Table 3 shows the mean of mice body temperature compared to room temperature measured from the difference of body temperature value and room temperature.

Mice are widely used as animal models in the laboratory to represent conditions in human, such as infection. Infection studies in human is very difficult to conduct, researchers instead often use mice as an experimental model due to several scientific reasons. Explanations that might be related to the results of this study is based on the calculation of limbs to body mass proportion. In human, extremities (whole arms and legs) constitute 50% of body mass, while in mice only account for 20% of body mass. Therefore, vasodilation in mice's tail causes great loss of heat. Vasomotor function is still essential to be considered. Knowledge on mice morphology and physiology is crucial to be understood before drawing any conclusions from studies about mice body temperature, especially when trying to extrapolate the data obtained from mice to be applied on human. Nowadays, the use of animal model for such study keeps on increasing. Scientific writings using mice were two-fold of those using rats (~27.00 vs 14.00 papers) (Gordon, 2017).

Body temperature measurements using non contact infrared thermometer were relatively easy, without the need of anesthesia and did not produce any injury to the animal. Infrared from the thermometer was fired at abdomen and anus regions. Available literature mentioned that

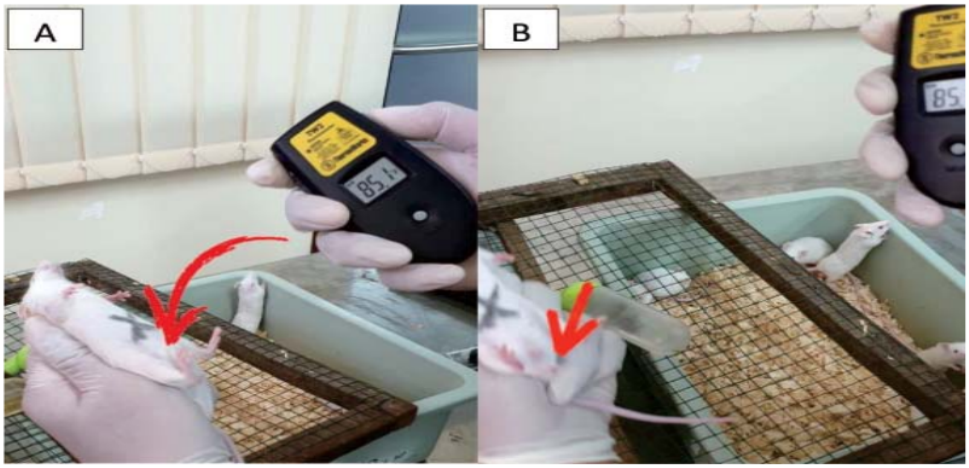

Figure 1. (A) Abdomen region and (B) Anal region for taking body temperature in mice

Table 1. Mice abdomen temperature (t_{abd}) and anal temperature (t_{an}) at 8th and 24th hour post-lipopolysaccharide (LPS) injection

Hour	Variable	LPS									
		Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6	Mice 7	Mice 8	Mice 9	Mice 10
8	Abdomen	36.90 °C	34.90 °C	36.60 °C	33.70 °C	32.70 °C	32.40 °C	33.50 °C	32.60 °C	32.10 °C	29.30 °C
	Anal	29.00 °C	29.30 °C	32.60 °C	29.80 °C	32.40 °C	24.90 °C	24.90 °C	23.40 °C	24.30 °C	27.50 °C
24	Abdomen	30.00 °C	29.50 °C	29.61 °C	33.66 °C	34.16 °C	33.00 °C	32.90 °C	31.27 °C	32.55 °C	34.66 °C
	Anal	27.16 °C	26.94 °C	26.72 °C	26.88 °C	26.88 °C	27.27 °C	24.88 °C	27.50 °C	30.66 °C	30.22 °C

Note: mice in control group were given NaCl 0.9% with equal volume as LPS

Table 2. Mice abdomen temperature (t_{abd}) and anal temperature (t_{an}) at 8th and 24th hour post-Natrium Chloride/NaCl 0.9% injection

Hour	Variable	NaCl									
		Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6	Mice 7	Mice 8	Mice 9	Mice 10
8	Abdomen	30.90 °C	29.30 °C	29.60 °C	28.60 °C	28.30 °C	28.50 °C	28.10 °C	29.60 °C	29.40 °C	29.00 °C
	Anus	26.30 °C	28.60 °C	28.00 °C	25.80 °C	27.50 °C	25.80 °C	26.80 °C	26.90 °C	27.80 °C	26.60 °C
24	Abdomen	32.80 °C	31.72 °C	30.60 °C	31.10 °C	32.66 °C	32.50 °C	31.83 °C	32.27 °C	32.66 °C	33.50 °C
	Anus	29.77 °C	26.27 °C	25.11 °C	26.88 °C	26.50 °C	25.94 °C	30.22 °C	29.38 °C	27.05 °C	26.83 °C

measurements using non-contact infrared thermometer can be done in mice at several regions, including: tympanic membrane, back, sternum, abdomen, and ano-genital areas, in which all of them refer to the core temperature (Mei *et al.*, 2018). Our data showed that measurements in anogenital area produced lower temperature than in abdomen region, which raised questions for the previous literature whether both abdomen and anogenital areas are truly reflective of core temperature. Explanations on this question should be further studied to achieve valid results which can then be applied on human.

Table 3. The mean of mice body temperature compared to room temperature

	Mean t_{abd}	Dt1	Mean t_{an}	Dt2
Control 8th hour	29.13°C	13.13°C	27.01°C	11.01°C
Treated 8th hour	32.47°C	16.47°C	27.81°C	11.81°C
Control 24th hour	32.12°C	16.12°C	27.51°C	11.51°C
Treated 24th hour	32.16°C	16.16°C	27.39°C	11.39°C
	Mean	15.47°C		11.43°C

Note: Room temperature is the temperature set at air conditioning room. Dt1: difference of t_{abd} with room temperature. Dt2: difference of t_{an} with room temperature.

Table 4. The difference between abdominal and anal temperature of the mice in control dan treatment group.

	Mean t_{abd}	Mean t_{an}	Dt_{abd} and t_{an}	T test
Control 8th hour	29.13°C	27.01°C	2.12°C	0.001
Treated 8th hour	32.47°C	27.81°C	4.66°C	0.009
T-test independent test	0.008	0.475		
Control 24th hour	32.12°C	27.51°C	4.614°C	0.000
Treated 24th hour	32.16°C	27.39°C	4.767°C	0.000
T-test independent test	0.475	0.882		

One of the most important contributing factors in core and surface temperature measurements in animals is the ambient temperature. The lower the ambient temperature, the lower the surface temperature, while core temperature will remain stable as long as the thermal regulation response still works normally. Another factor which might affect the surface temperature measurements is the condition of mice restraining device. Surface temperature increased after the restraining device was applied to the mice. Stress in mice will activate sympathetic nervous system, leading to thermogenesis and vasocontrictions of skin vessels, resulting in differences in core and surface temperature (Kawakami *et al*., 2018). In regards to the data obtained in this study, we still cannot describe clearly. The measurement of body temperature at anus area (t_{an}) resulted in lower result than that of abdomen area (t_{abd}). This might be of influence of vasodilation in the mice's tail. Lipopolysaccharide (LPS), a bacterial endotoxin, can cause vasodilation (Gunnett *et al*., 1998; Virzì *et al*., 2017).

The difference between mean t_{abd} with the room temperature was relatively high (15.48°C). This might be due to vasoconstrictions in abdomen area as compared to the anal. The difference between mean t_{an} with the room temperature was 11.43°C. This suggests that mice's blood vessels in the anal were experiencing vasodilation as compared to the abdomen. This wide gap between mice body temperature and room temperature might indicate the exceptional of mice to be capable of withstanding extreme temperature stimulations. The surface temperature can be explained by thermoregulation role of the skin and changes in vasomotor tones as compensations of heat changes (Newsom *et al*., 2004). The mice metabolism also affects the value of body temperature (Škop *et al*., 2020). Based on the results of this study, the choice of area for measuring body temperature in mice is a strong consideration in addition to the problem of central or surface body temperature, and also metabolism.

CONCLUSION

Temperature measurement at anal area using non-contact infrared thermometer showed lower results as compared to that of at abdomen. Several factors lead to the mice body temperature changes as shown in this study are including room temperature, LPS injection, and the area of measurement.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6