

## THE EFFECT OF 3000 MG VITAMIN C INFUSION ON HISTAMINE LEVEL OF SEVERELY BURNT PATIENTS

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### ABSTRAK

*Proses patologi pada luka bakar mengakibatkan inflamasi lokal dan sistemik yang menyebabkan perpindahan cairan intravaskular menuju ruang interstitial. Hal itu disebabkan karena perubahan permeabilitas vaskuler diantaranya karena adanya peningkatan histamin. Efek anti inflamasi vitamin C adalah melalui perusakan struktur cincin imizadole dari molekul histamin. Tujuan dari penelitian ini adalah untuk mengetahui efek pemberian infus vitamin C 3000 mg terhadap kadar histamine darah pada pasien dengan luka bakar berat di unit luka bakar Dr. Soetomo Hospital, Surabaya dari bulan Juni-Desember 2012 (usia 16-65 tahun, kriteria luka bakar dari American Burnt Association, 2007). 16 pasien dibagi menjadi 2 kelompok; G1 (kontrol, diberi infus vitamin C 2x400 mg per hari selama 72 jam) dan G2 (vitamin C 3000 mg per hari selama 72 jam). Variable yang diperiksa adalah kadar histamine jumlah sel darah putih, fungsi ginjal dan blood gas analysis (BGA). Data dianalisis dengan tes t dan tes t berpasangan (signifikan pada  $p < 0,05$ ). Kadar histamine G1 lebih tinggi dibanding G2 ( $p < 0,05$ ). Tidak ada beda signifikan pada analisis fungsi ginjal dan BGA antara G1 dan G2. Jumlah sel darah putih G2 lebih rendah dibanding G1 ( $p = 0,045$ ). Pemberian infus vitamin C dosis tinggi dapat menurunkan kadar histamin darah pada pasien luka bakar berat. (FMI 2014;50:96-99)*

**Kata Kunci:** vitamin C, histamin, pasien luka bakar parah

### ABSTRACT

*The pathology process in burn injury is both a local and systemic inflammatory reaction, the end result of which is an almost immediate shift of intravascular fluid into the surrounding interstitial space. This occurs as a consequence of changes in vascular permeability as the normal capillary barrier is disrupted by a host of mediators, one of them is histamine. Vitamin C functions as an anti-inflammatory on burn by destroying imizadole ring structure of the histamine molecule. The purpose of this research is to determine the effect of 3000 mg vitamin C infusion on histamine level in severely burnt patients. A randomized pre & posttest control group design. Histamine levels were determined in 16 severely burned patients in burn injury unit of Dr. Soetomo Hospital, Surabaya from June-December 2012 (age 16-65 years old, American Burn Association criteria, 2007). 16 patients were divided into 2 groups; G1 (control, given infusion of vitamin C 2x 400 mg per day for 72 hours) and G2 (vitamin C 3000 mg per day for 72 hours). Variable examined were histamine level of leucocyte, renal function, and blood gas analysis (BGA). Data were analyzed with t test and coupled t test (significant at  $p < 0.05$ ). G1 histamine level was higher than G2 ( $p < 0.05$ ). There were no significant results from renal function analysis and BGA between G1 and G2. The amount of G2 leucocyte was lower than G1 ( $p = 0.045$ ). Giving vitamin C infusion with high dose is able to decrease blood histamine level in severely burnt patient. (FMI 2014;50:96-99)*

**Keywords:** vitamin C, Histamine, severely burned patients

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### INTRODUCTION

Burn injury remains a big problem causing high morbidity and mortality. Incidence of burn injury in U.S was 2 million people in a year, with 50.000-100.000 patients were indicated for hospitalization (Monafo 1996, Jordan & Harrington 1997). In Burn Unit of Dr. Cipto Mangunkusumo Hospital Jakarta, there were 107 patients with 37.38% morbidities in 1998. In Burn Unit of Dr. Soetomo Hospital Surabaya, there were 167 cases, 94 cases of extensive burns, and mortality on 11 patients. The management is very difficult, time

consuming, and high cost. Medical team often has bad experiences to rehabilitate burned patients until they are going back to their community. A research by Tanaka et al (2000) suggest adjuvant administration of high-dose ascorbic acid (66 mg/kg) during the first 24 hours after thermal injury significantly reduces resuscitation fluid volume requirements, body weight gain, and wound edema. A reduction in the severity of respiratory dysfunction was also apparent in these patients. However, this occurs as a consequence of changes in vascular permeability as the normal capillary barrier is disrupted by a host of mediators, one of them is

histamine. Vitamin C functions as an anti-inflammatory on burn by destroying imidazole ring structure of the histamine molecule. Recent researches, high dose of vitamin C (66 mg/kg/day) could reduce the resuscitation fluid in burned patients, another research in seriously ill and injured patients; 3000 mg/day of vitamin C should be given for at least the first 3 days. In this research, we examine the histamine plasma level in severely burned patients after being treated by administration of 3000 mg/day vitamin C for three days consecutively. The purpose of this research was to review the effect of 3000 mg vitamin C infusion on histamine level in severely burned patients. In addition, histamine level is an important indicator in this research.

## MATERIALS AND METHODS

The samples were 16 severely burned patients (age 16-65 years old) treated at the Burn Unit of Dr. Soetomo Hospital in Surabaya from June - December 2012 fulfilling the inclusion criteria. The inclusion criteria were the age between 16-65 years old, thermal injury not more than 24 hours after trauma, and patient agrees to become participant. Meanwhile, the exclusion criteria were patient refuses to be participant, previous history of diabetes mellitus, and renal dysfunction. Patients would be randomized prospectively into a control (Group 1=G1) and an ascorbic acid group (Group 2=G2). Randomization was performed according to the month of admission. The profiles of both groups were similar regarding age, sex, body weight, type of thermal injury, percentage of TBSA, percentage of full thickness burn, and the presence of smoke inhalation.

Severely burned patient, who had been resuscitated and fulfilled the inclusion criteria, the blood sample of histamine was taken (@ 3cc on 24 hours after resuscitation. Then we measured the level of histamine, leukocyte, renal function test, blood gas analysis, and urine production/ hour. The control group (G1), only received vitamin C 2x400 mg/day. The ascorbic acid group (G2) received 3000 mg of vitamin C. The ascorbic acid solution, which was covered with black paper to prevent auto-oxidation by sunlight, was administered as a continuous intravenous infusion by pump for 3 consecutive days. On 72 hours after resuscitation, the blood sample was taken again to measure the same indicators as mentioned above.

The independent variable was vitamin C 3000 mg infusion, given for three days consecutively. The dependent variable was histamine serum level on 24 hours post resuscitation and 72 hours post resuscitation. Principle of the test first, histamine was quantitatively acylated. The subsequent competitive ELISA kit uses

the microtiterplate format. The antigen was bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte competed for a fixed number of antiserum binding sites. After the system was in equilibrium, free antigen and free antigen-antiserum complexes were removed by washing. The antibody bound to the solid phase was detected by an anti-rabbit IgG peroxidase conjugate using TMB as a substrate. The reaction was monitored at 450 nm. Quantification of unknown samples was achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. The blood sample was put in the cold plastic tube, contains EDTA (10mM). Stored in 2-8°C, could last for 6 hours. The sample was centrifuged for 10 minutes, only the upper part of the plasma was used to measure the histamine level. To compare the histamine changes in pretreatment and post treatment between the two groups, normalization test was used. If the data result was normal, t 2 free sample test, on the contrary if the result was not normal, Mann Whitney test was used.

## RESULTS

The data of histamine level were analyzed using Kolmogorov-Smirnov one sample test and the results were normal distribution, then the data were analyzed using analysis of variant method (ANOVA). The data of WBC, BUN, creatinine serum, and Blood Gas Analysis were analyzed using paired t-test. The total patients were 10. The mean of histamine level on treatment group on day-1, day-2, and day-4 after resuscitation were stable. Slight increase was found on day-2. Histamine level in control group was lower on day-1 and day-2 after resuscitation compared to treatment group. Histamine level in control group was highly elevated on day-4 after resuscitation, whereas on the two observations before, the histamine level was stable (Figure 1).

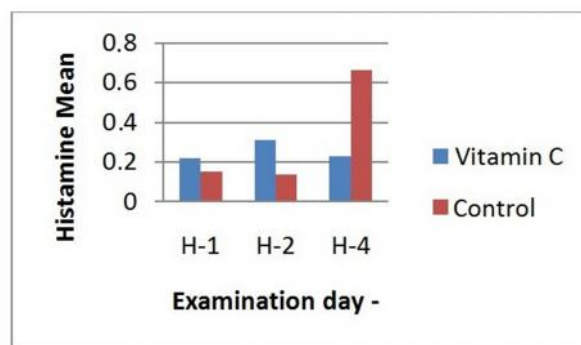


Figure 1. Comparison of histamine mean between treatment group and control group on day-1, day-2, and day-4.

The decreasing of WBC means before and after in control group was not significant with  $p > 0.05$  (Figure 2). Statistically significant of decreasing WBC mean were found in treatment group ( $p < 0.045$ ). The mean of BUN were increased in treatment and control group, but not statistically significant ( $p > 0.05$ ). Creatinine serum mean between treatment and control group were elevated non-significantly (Figure 2 and 3).

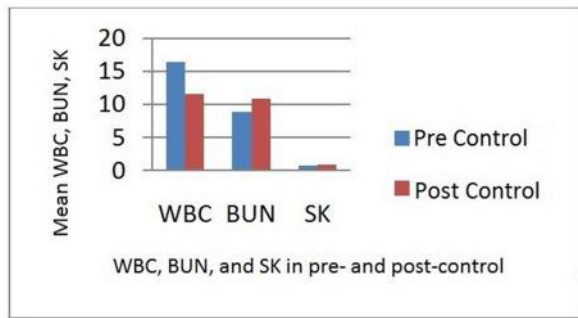


Figure 2. The mean of WBC, BUN, and serum creatinine in pre and post control group.

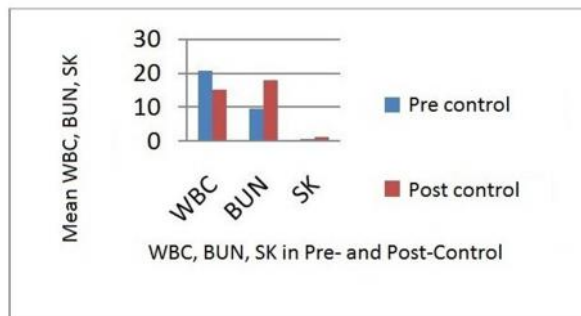


Figure 3. The mean of WBC, BUN, creatinine serum in pre and post treatment of 3000 mg vitamin C

The mean result of pH, HCO<sub>3</sub>, and BE were almost the same between the two groups. SO<sub>2</sub> mean were more decreasing in control group compare to treatment group. On the contrary pCO<sub>2</sub> mean in treatment group were higher than control group (Figure 4). A steep decreased of pO<sub>2</sub> mean could be seen in treatment group than control group (Figure 5).

The BUN and Thrombocyte level were within normal limit. On the contrary, creatinine level and WBC there was one patient who died because of MODS have creatinine level of 1.5 and WBC level 22. Paired sample test was used in placebo group. Sig. (2-tailed) values of less than .05 were accepted as significant. There were no significant result between pre-post treatment in BUN sig. (2 tailed) 0.626, creatinine Level sig. (2 tailed) 0.117, WBC sig. (2 tailed) 0.483, and Thrombosis sig. (2 tailed) 0.201, in Placebo Group. There were 2 patients with metabolic acidosis. The BGA result

became better after fluid resuscitation. Table 2b illustrates Blood Gas Analysis using paired sample test in placebo group. Sig. (2-tailed) values of less than .05 were accepted as significant. PH sig. (2-tailed) 0.074, PCO<sub>2</sub> sig. (2-tailed) 0.325, pO<sub>2</sub> sig. (2-tailed) 0.936, HCO<sub>3</sub> sig. (2-tailed) 0.547, BE sig. (2-tailed) 0.762, SO<sub>2</sub> sig. (2-tailed) 0.379. There was no significant result between pre-post treatments in Blood Gas Analysis, in Placebo Group.

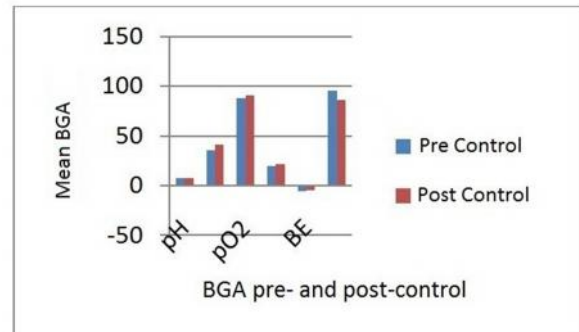


Figure 4. BGA mean in pre and post control

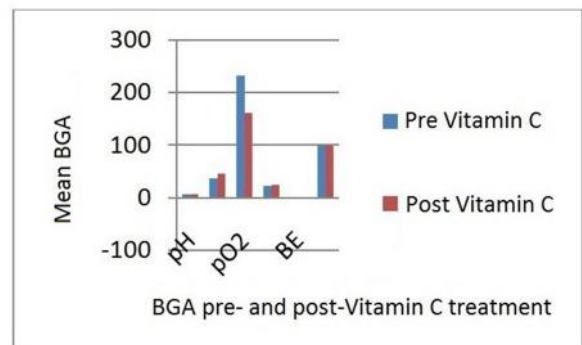


Figure 5. BGA mean in pre and post treatment

The only abnormal BUN result was 166 in patient with 50% TBSA, eventually this patient died due to MODS (table 3a). Creatinine level were raised in 2 patients 2.48 and 2.68. The second patient was diagnosed electrical injury high voltage. His creatinine level was gradually decreasing in later examination. In the other three patients, creatinine serum level were normal. The result of WBC in 2 patients were high, 27 and 30, 48 respectively. The last three patients were decreasing. One Patient B with pancytopenia the thrombocyte result was 78. Statistical analysis of BUN, creatinine serum, WBC and thrombocyte in pre and post-vitamin C infusion group were described in Table 3. Paired sample test was used in placebo group. Sig. (2-tailed) values of less than .05 were accepted as significant. There were no significant result between pre-post treatment in BUN sig. (2-tailed) 0.368, creatinine serum sig. (2-tailed) 0.361, and Thrombosis level sig. (2-tailed) 0.63, in

vitamin C infusion Group. The only significant result was in WBC level sig (2-tailed) 0.045. There was metabolic acidosis in one patient.

## DISCUSSION

The pathophysiology of the burn patient shows the full spectrum of the complexity of inflammatory response reactions. In the acute phase, inflammation mechanism may have negative effects because of capillary leak, the propagation of inhalation injury and the development of multiple organ failure (Evers et al 2010). Burn wound has a direct effect on mast cells, promoting histamine secretion that would increase the activity of xanthine oxidase and the production of Reactive Oxygen Species (Santos et al 2000). Previous researches have indicated that this developing hypovolemia and immediate need for fluid resuscitation appear to be related to the increased vascular permeability and capillary leakage observed physiologically and morphologically after thermal injury. Recent researches also suggest that reactive oxygen species (ROS) generated by thermal injury are involved in the increased micro vascular permeability, edema formation, and tissue damage associated with burn. ROS have been implicated in much of the complex pathophysiologic processes after burn that are characterized by an inflammatory response that activates neutrophils, stimulates complement, induces intravascular hemolysis, and releases vasoactive substances, pro inflammatory cytokines, catabolic hormones, and other inflammatory mediators such as histamine, bradykinin, and prostaglandins (Hatherill et al 1986). Oxygen radicals may play an important role in injury due to thermal burns. Friedl et al (1989) suggest the releasing of histamine by mast cells after burns, increasing xanthine oxidase activity, in turn, producing free oxygen radicals. Another

Major burn causes inflammation, whereas white blood cells were produced to protect against it. Inflammation regulates local and systemic mediator and one of the local mediator is histamine. Vitamin C could reduce the histamine level by destroying the imidazole ring in histamine, causing a decrease in histamine level. A

decrease in histamine level, reduce the inflammation. Therefore the white blood cells amount also decreased.

## CONCLUSION

The effect of 3000 mg vitamin C infusion on histamine level in severely burned patients is acting as an ant inflammation by regulating the histamine level. There are no significant results on blood gas analysis, BUN, creatinine serum. The significant result of leukocyte could be seen in patients with vitamin C infusion.

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## REFERENCES

- Evers LH, Bhavsar D, Mailänder P (2010). The biology of burn injury. *Exp Dermatol* 19, 777-783
- Friedl HP, Till GO, Trentz O, Ward PA (1989). Roles of histamine, complement and xanthine oxidase in thermal injury of skin. *Am J Pathol* 135, 203-217
- Hatherill JR, Till GO, Bruner LH, Ward PA (1986). Thermal injury, intravascular hemolysis, and toxic oxygen products. *J Clin Invest* 78, 629-636
- Jordan BS and Harrington DT (1997). Management of the burn wound. *Nurs Clin North Am* 32,251-273
- Monafo WW (1996). Initial management of burns. *N Engl J Med* 335,1581-1586
- Santos FX, Arroyo C, García I, Blasco R, Obispo JM, Hamann C, Espejo L (2000). Role of mast cells in the pathogenesis of postburn inflammatory response: reactive oxygen species as mast cell stimulators. *Burns* 26, 145-147
- Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S (2000). Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: a randomized, prospective study. *Arch Surg* 135, 326-331