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Response of HIV seropositive red blood cells to oxidative stress is no less than that of normal red blood cells: A preliminary report

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Objective : *To study response to oxidative stress of the red blood cells of the HIV seropositive people.*

Subjects : *Nine EDTA blood samples taken from anti HIV seropositive patients and nine control blood samples.*

Setting : *Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University.*

Methods : *We used in vitro model of study of oxidative stress, purposed by a previous study as the tool employed in this study. Briefly, 0.1 mL of blood sample was added by the acetylphenylhydrazine (100 mg %) 2 mL. Then it was incubated at 37 Degree Celsius for 2 hours. The product was smeared and manually microscopic assessed for Heinz bodies per 100 red blood cells. The rate of Heinz bodies was accepted as the level of response to oxidative stress. Here we performed this in vitro study on all collected blood samples.*

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Result : *We found that all blood samples presented 100 % rate of Heinz body. There was no significant difference ($p > 0.05$) between that of the anti HIV seropositive group and the control. Also, among the anti HIV seropositive group, there was no significant difference ($p > 0.05$) between the subjects with CD4+ > 500 and CD4+ < 500 μ/L.*

Conclusion : *From this preliminary study, we concluded that the response of the anti HIV seropositive red blood cell to oxidative stress is not less than that of red blood cells in normal subjects. Furthermore, no difference was detected between the early and the late stage of HIV infection. Nevertheless, the observers notified more aberrant in the shape of red blood cells in the anti HIV seropositive group. However, further study to determine the total antioxidant is strongly recommended.*

Keywords : *Oxidative stress, HIV.*

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แจ่มใส สุวรรณศักดิ์ศรี, วิโรจน์ ไววานิชกิจ, สุพรรณ สุขอรุณ, จิตรลดา ประชาศิลป์, อัจฉราพร โชติพืช. การตอบสนองของเม็ดเลือดแดงในคนติดเชื้อ HIV ต่อสภาวะเครียด oxidative ไม่ต่ำกว่าคนปกติ : รายงานการศึกษาเบื้องต้น. จุฬาลงกรณ์เวชสาร 2545 พ.ย;46(11): 901 - 6

วัตถุประสงค์ : *ศึกษาการตอบสนองของเม็ดเลือดแดงในคนติดเชื้อ HIV ต่อ สภาวะเครียด oxidative*

ตัวอย่าง : *เลือด EDTA จากผู้ติดเชื้อ HIV 9 ราย และเลือดควบคุม 9 ราย*

สถานที่ทำการศึกษา : *ภาควิชาเคมีคลินิก คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

วิธีการศึกษา : *ได้ทำการศึกษาโดยใช้แบบทดลองที่มีได้มีการรับรองไว้แล้ว โดยใช้ตัวอย่างเลือด 0.1 mL เติมด้วย acetylphenylhydrazine (100 mg %) 2 mL แล้ว incubate ที่ 37 องศาเซลเซียส 2 ชั่วโมง นำสารผลิตภัณฑ์ไปทำ smear และนับ Heinz bodies ต่อ เม็ดเลือดแดง 100 เซลล์ด้วยกล้องจุลทรรศน์ ใช้อัตราของ Heinz bodies เป็นเครื่องชี้วัดการตอบสนองต่อสภาวะเครียด oxidative โดยได้ใช้แบบแผนการทดลองนี้กับทุกตัวอย่าง*

ผลการศึกษา : *ทุกตัวอย่างให้อัตราการเกิด Heinz body 100 % ไม่พบความแตกต่างระหว่างกลุ่มติดเชื้อและไม่ติดเชื้อ HIV และไม่พบความแตกต่างระหว่างกลุ่มติดเชื้อ HIV ที่ CD4+ > 500 และ CD4+ < 500 μ/L*

สรุป : *จากการศึกษาเบื้องต้นนี้พบว่า การตอบสนองของเม็ดเลือดแดงในคนติดเชื้อ HIV ต่อสภาวะเครียด oxidative ไม่ต่ำกว่าคนปกติ ไม่พบความแตกต่างระหว่างผู้ติดเชื้อ HIV ระยะแรกและระยะหลัง อย่างไรก็ตามผู้วิจัยพบความผิดปกติของรูปร่างของเม็ดเลือดแดงในกลุ่มผู้ติดเชื้อ HIV การศึกษาต่อไปโดยวัดระดับ total antioxidant เป็นสิ่งที่จำเป็น*

คำสำคัญ : *สภาวะเครียด oxidative, HIV*

Human immunodeficiency virus (HIV) infection, a worldwide infection, is a serious problem in the present day. It is characterized as the breakdown of the immune system due to the decrease of selected cells in the system. The results of the decrease are defects in immune function which allows "opportunistic" infections that normally do not infect people who have healthy immune system to be vulnerable to infects and bring them to lethal ends.

Muscle loss is a common complication in people living with HIV/AIDS. When muscle mass of a person significantly decreases, the term "wasting" is often applied. [1] Researches and clinical experiences have shown that people who experience the wasting have a lower survival rate than those who are able to maintain their body weight.

Some researchers proposed that oxidative mechanisms are crucial in the pathogenesis of AIDS (acquired immune deficiency syndrome). A prediction of the hypothesis was that the mechanisms responsible for AIDS could be reversed by the administration of reducing agents, especially those containing sulphydryl groups (SH groups). The discovery of HIV resulted in a broadening of the hypothesis that oxidative stress is a principal mechanism in both the development of AIDS and expression of HIV.[2-3]

However, the general acceptance of the hypothesis of HIV/AIDS completely overshadowed the alternative hypothesis. Although many scientists have questioned the role of HIV in the causation of AIDS,[4] most researchers on AIDS consider HIV the sole "*sine qua non*" cause of AIDS. On the other hand, recent empirical observations from three seemingly unrelated areas of AIDS research are in agreement with the hypothesis that oxidative mechanisms play a critical role in HIV expression and the development of AIDS. To help prevent and treat "wasting syndrome" in AIDS patients, many people are dependent on nutritional supplements. [5]

Here we reported an interesting result from our preliminary *in vitro* study that the response to the oxidative stress of blood taken from normal population and AIDS patients were not different.

Materials and Methods

The study was performed as a cross sectional study. All experiments were conducted at the Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University. All analyses were performed on the room temperatures by the same observers.

Sample selection

Nine anti-HIV seropositive patients (5 males and 4 females) were selected into the study. Five milliliters of EDTA blood specimen left over from routine analysis for complete blood count (CBC) of each subject was used for investigation . All subjects were non-anemia according to their CBC findings. Also, we selected nine control blood samples from anti HIV seronegative subjects.

In vitro study of oxidative stress

We used *in vitro* model of study of oxidative stress purposed in a previous study [6] for this study. Briefly, 0.1 mL of blood sample was added by the acetylphenylhydrazine (100 mg %) 2 mL. Then it was incubated at 37 Degree Celsius for 2 hours. The product was smeared and manually microscopic

assessed for Heinz bodies per 100 red blood cells.[6] The rate of Heinz bodies was accepted as the level of response to oxidative stress. Here we performed this *in vitro* study on all collected blood samples.

Statistical analysis

The rate of Heinz body in each blood sample was recorded. Then the average values of the HIV seropositive group and the control group were calculated and compared using F test for statistical significance level ($p = 0.05$).

Result

Interestingly, we found all blood samples presented 100 % rate of Heinz body. There was no significant difference ($p > 0.05$) between the rate found among the anti HIV seropositive group and the control . Also, among the anti HIV seropositive group, there was no significant difference ($p > 0.05$) between the subjects with CD4+ > 500 and CD4+ < 500 μ/L (Table 1).

Discussion

Recently it has been proposed that reactive oxygen species (ROS) are involved in the pathogenesis of many human diseases. An elevated level of these molecules causes oxidative stress which is toxic for living cells. Oxidative stress is the cause of many damages of cellular structures, as a result of free radical reactions with proteins, lipids, nucleic acids, etc. In most human diseases, overproduction of ROS is characteristic for early stage of disease.[7-8]

Some of infectious factors, e.g. viruses, can cause oxidative stress by disturbance of cellular antioxidants system, or induction of oxidative reactions. There is some evidence of presence of oxidative stress in early stage of HIV infection (glutathione and other antioxidants loss in serum and decreased activity of antioxidant enzymes). All these metabolic disturbances may involve in the pathogenesis of AIDS, for example through incorrect induction of lymphocyte apoptosis, tumors related to AIDS and high rate of HIV mutation. [2-3] This possibly suggests an important role of oxidative stress in the pathogenesis of AIDS and that the administration of antioxidant drugs, by HIV infected patients,[5] may offer protection against mechanisms responsible for lymphocyte apoptosis and AIDS related carcinogenesis.

Here, we performed an *in vitro* study to determine whether the response of red blood cells of anti HIV seropositive patients to oxidative stress is different from that of the anti HIV seronegative subjects. Interestingly, we found no difference, all samples

Table 1. Average rate of Heinz bodies in the studied blood samples.

Group	Averagerate of Heinz bodies (%)	Deformed red blood cell
Anti HIV seronegative (n = 9)	100	No
Anti HIV seropositive (n = 9)	100	Yes
❑ CD4+ > 500 μ/L (n = 3)	100	Yes
❑ CD4+ < 500 μ/L (n = 6)	100	Yes

showed 100 % response to the oxidative induced agent. From this preliminary study, we concluded that the response of the anti-HIV seropositive red blood cells to oxidative stress is no less than that of the red blood cells of normal people. Furthermore, there was no difference detected between early and late stage of HIV infection. Nevertheless, the observers notified more aberrant in the shape of red blood cells in the anti-HIV seropositive group. However, this study is only a preliminary study; hence, further study in a larger group of subjects, is strongly recommended.

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