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Effect of Ramadan Fasting on Endorphin and Endocannabinoid level in Serum, PBMC and Macrophage

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ABSTRACT: *Ramadan is a holy month and Muslims fast during this month that will be altered daily life pattern from diurnal to nocturnal in which its can be as stressor and the body will response to this stressor. Endorphin and endocannabinoid is effectors of stress, so the aim of this study was to determine the effect of Ramadan fasting (RF) as stressor on endorphin and endocannabinoid of serum, PBMC and macrophage to response stressor. Twenty seven healthy volunteers male aged 18–22 years (mean±SD 20.26±1.13 years) who fasted during Ramadan participated in the study. Blood sampling was conducted on 7 days before Ramadan, days 7 and 21 of Ramadan. The following were measured by enzyme-linked immunosorbent assay (ELISA) method: endorphin and endocannabinoid in serum, PBMC and macrophage. Endorphin serum and PBMC increase significant ($p<0.05$) on day 7 and 21 of Ramadan compared to before Ramadan. Day 7 compared with day 21 was increase significant on serum and decrease significant on PBMC. Endorphin macrophage increase not significant ($p>0.05$) on day 7 but significant on day 21 of Ramadan compared to before Ramadan and day 7 compared with day 21 was significant too. Endocannabinoid serum and PBMC increase significant ($p<0.05$) on day 7 and 21 of Ramadan compared to before Ramadan but day 7 compared to day 21 was not significant ($p>0.05$) on serum and decrease not significant on PBMC. Endocannabinoid macrophage decrease not significant ($p>0.05$) on day 7 but significant on day 21 of Ramadan compared to before Ramadan and day 7 compared to day 21 was decrease significantly. Before Ramadan endorphin level highest was in serum and lowest in macrophage, day 7 highest was in PBMC and lowest in serum and day 21 highest was in serum and lowest in PBMC, in which each other was significantly difference. Endocannabinoid level before Ramadan, day 7 and day 21 was had same pattern, highest in serum and lowest in macrophage, each other was significantly different. The results obtained indicate that subject were tolerance during RF. The serum, PBMC and macrophage had difference stress responses to RF as stressor. Response stress of serum higher than PBMC and macrophage, while PBMC higher in early and macrophage in the end of RF. Are the serum, PBMC and macrophage during RF in distress or eustress condition and are during culture process contributing to response stress of macrophage still unelucidated.*

Keyword: *Ramadan fasting, stress, stressor, endorphin, endocannabinoid, serum, PBMC, macrophage*

I. INTRODUCTION

One of the 5 fundamental rituals of Islam is fasting during the month of Ramadan and it's a great opportunity for scientific research due to its peculiar nature [1]. Every year, millions of Muslims fast from dawn until sunset during the lunar month of Ramadan. Ramadan can occur in any of the four seasons and the hours spent fasting vary accordingly, from 11 hours to 18 hours (average 13 hours) a day [2]. The Ramadan fasting (RF) period is associated with altered meal patterns, sleep and ritual habits in Muslims [3], in which it's will change a circadian rhythm of the body from diurnal to nocturnal and it's become stressor and the body will response to this stressor [4]. Stress consist of stress perception and stress response [5].

We examined the effect of fasting during the lunar month of Ramadan as stressor on endorphin and endocannabinoid level as effectors stress at serum, PBMC in a group of 14 healthy males and macrophage in a group of 27 healthy males. The main emphasis of this study was to elucidate the stress response on serum, PBMC and macrophage during RF, as little is known about this topic.

Stress describes the capacity and mechanisms to sustain and adjust for externally or internally challenging situations [6]. The stress response is subserved by the stress system, which is located both in the central nervous system and the periphery [7]. Therefore, organisms can rely on the endogenous ability to self-regulate stress and stressors, i.e., autoregulatory stress management. [6]. The principal effectors of the stress system include corticotrophin-releasing hormone (CRH); arginine vasopressin; the proopiomelanocortin-derived peptides α-melanocyte-stimulating hormone and beta-endorphin, the glucocorticoids; the catecholamines norepinephrine and epinephrine [7]. There seems to exist a common neurobiological mechanism, i.e., limbic autoregulation, that involves dopamine, morphine and other endogenous signalling molecules, e.g., other opioid receptor agonists, endocannabinoids, oxytocin or serotonin, many of which act via Nitric Oxide ((NO) release, and this share seems to be of critical importance for the self-regulation and management of stress: stress management is an endogenous potential [6].

Endocannabinoid signalling within stress-sensitive nuclei of the hypothalamus, as well as upstream limbic structures such as the amygdala, suggests it may play an important role in regulating the neuroendocrine and behavioral effects of stress, that endocannabinoid signalling is involved in both activating and terminating the hypothalamic-pituitary-adrenal (HPA) axis response to both acute and repeated stress. In addition to neuroendocrine function, however, endocannabinoid signalling is also recruited by stress and glucocorticoid hormones to modulate cognitive and emotional processes such as memory consolidation and extinction. Collectively, these data demonstrate the importance of endocannabinoid signalling at multiple levels as both a regulator and effectors of the stress response [8].

The beneficial claims of fasting are supported by experimental research, which has found fasting to be associated with increased brain availability of serotonin, endogenous opioids, and endocannabinoids. Fasting-induced neuroendocrine activation and mild cellular stress response with increased production of neurotrophic factors may also contribute to the mood enhancement of fasting [9].

II. MATERIAL AND METHODS

Blood samples were drawn for examination of beta endorphin and endocannabinoid level from all participant and samples were drawn between 9 am and 12 noon to avoid diurnal variation at 7 day before, day 7 and day 21 of Ramadan fasting.

Five milliliters of venous blood from the antecubital vein were collected in an ethylenediaminetetraacetic acid (EDTA) vial. The plasma was separated and kept at −80°C until analysis [10]. Peripheral blood mononuclear cells (PBMC) were isolated using dextran sedimentation and Hypaque-Ficoll density-gradient separation followed by hypotonic lysis of erythrocytes as previously described [11]. Human monocytes were isolated from buffy coats and cultured for 6 days in 24- or 6-well tissue culture plates (Corning, Corning, NY) as previously described [12], with a minor modification: cells were cultured in RPMI 1640 with Penstrep 1 % (Sigma Aldrich), serum free Nabic 0.2 % (Bio World) and FBS 10 % (Gibco Inc), pH 7,2 incubate 37° C, 5% CO2. Culture medium and nonadherent cells were removed by aspiration every 3 days of culture, and monolayers were subsequently incubated with fresh culture medium supplemented with 10% autologous serum [13].

Purified PBMC and macrophage were each resuspended in incubation buffer (sterile pyrogen-free Hanks balanced salt solution [HBSS; Bio-Whittaker], vortexed and centrifuged 1400 rpm 7 minute 4°C. Pellets add with RIPA+PIC+PMSF. Vortexed and incubation on ice for 30 minute. Centrifuge 12.000 rpm for 20 minute at 4°C. Supernatant kept on -20°C until analyze. Beta endorphin and endocannabinoid level was estimated duplo by enzyme linked immunosorbent assay using Endorphin, beta (Human) - EIA Kit (Uscn Life Science Inc. E90806Hu) and endocannabinoid using BioQuant kit (BQ205-096).

Statistical Analysis: One-Sample Kolmogorov-Smirnov Test, mean, ANOVA and Duncan test were performed using SPSS version 11.5 for Windows.

III. RESULTS

The study population consisted of healthy males, medical student in medical school at Brawijaya University. Due to various limitations, including the needs for laboratory examinations, fresh blood to be examined immediately, and adequate blood volumes, most blood samples were unsuitable, leaving only 14 cases eligible for endorphin and endocannabinoid of Serum and PBMC and 27 cases for endorphin and cannabinoid of macrophage. The subjects were aged 18–22 years (mean±SD 20.26±1.13 years).

Our result showed serum endorphin before Ramadan (mean±SD 3675.607 ± 3016.7269), day 7 (mean ± SD 6092.571±1737.4132), and day 21 (mean ± SD 7884.179 ± 769.2313), ANOVA approximately zero, with Duncan test before Ramadan compare with day 7 and day 21 were significant ($p < 0.05$), day 7 compare with day 21 also significant ($p < 0.05$). Endorphin of PBMC before Ramadan (mean±SD 1951.143±1031.3200), day 7 (mean ± SD 3744.714 ± 1383.0132), and day 21 (mean±SD 3219.714± 1470.6927), ANOVA $p = 0.003$ with Duncan test day 7 and day 21 compare with before Ramadan were significant ($p < 0.05$), day 7 compare

with day 21 not significant ($p > 0.05$). Endorphin of makrofag before Ramadan (mean±SD 1872.374±601.6360), day 7 (mean±SD 1828.719± 650.9395), and day 21 (mean±SD 3468.074± 1550.5873), ANOVA $p = 0.000$ with Duncan test day 7 were not significant ($p > 0.05$), day 21 significant ($p < 0.05$) compare with before Ramadan and day 7 compare with day 21 were significant ($p < 0.05$).

Sampel	N	Mean± SD	ANOVA	Duncan test
Serum Endorphin				
Before Ramadan	**14**	**3675.607 ± 3016.7269**		**1. p < 0.05 ***
Day 7	**14**	**6092.571±1737.4132**	**p = 0.000**	**2. p < 0.05***
Day 21	**14**	**7884.179± 769.2313**		**3. p < 0.05***
Serum endocannabinoid				
Before Ramadan	**14**	**27.557±0.4345**		**1. p < 0.05 ***
Day 7	**14**	**27.900±0.3211**	**p = 0.003**	**2. p < 0.05***
Day 21	**14**	**28.071±0.3604**		**3. p > 0.05**
PBMC Endorphin				
Before Ramadan	**14**	**1951.143±1031.3200**		**1. p < 0.05***
Day 7	**14**	**3744.714±1383.0132**	**p = 0.000**	**2. p < 0.05***
Day 21	**14**	**3219.714± 1470.6927**		**3. p < 0.05***
PBMC endocannabinoid				
Before Ramadan	**14**	**8.643±1.8084**		**1. p < 0.05***
Day 7	**14**	**15.421±1.7533**	**p = 0.000**	**2. p < 0.05***
Day 21	**14**	**13.621±3.7785**		**3. p > 0.05**
Macrophage Endorphin				
Before Ramadan	**27**	**1872.374±601.6360**		**1. p > 0.05**
Day 7	**27**	**1828.719± 650.9395**	**p = 0.000**	**2. p < 0.05***
Day 21	**27**	**3468.074± 298.4107**		**3. p < 0.05***
Macrophage endocannabinoid				
Before Ramadan	**27**	**2.848±0.4154**		**1. p > 0.05**
Day 7	**27**	**2.833±0.2717**	**p = 0.000**	**2. p < 0.05***
Day 21	**27**	**2.622±0.3598**		**3. p < 0.05***
Endorphin before Ramadan				
Serum	**14**	**3675.607±3016.7269**		**1. p < 0.05***
PBMC	**14**	**1951.143±1031.3200**	**p = 0.000**	**2. p < 0.05***
Makrofag	**27**	**1872.374±601.6360**		**3. p > 0.05**
Endocannabinoid before Ramadan				
Serum	**14**	**27.557±0.4345**		**1. p < 0.05***
PBMC	**14**	**8.643±1.8084**	**p = 0.000**	**2. p < 0.05***
Makrofag	**27**	**2.848±0.4154**		**3. p < 0.05***
Endorphin day 7				
Serum	**14**	**6092.57±464.3432**		**1. p < 0.05***
PBMC	**14**	**3744.71±369.6258**	**p = 0.000**	**2. p < 0.05***
Makrofag	**27**	**1828.72±125.2734**		**3. p < 0.05***
Endocannabinoid day 7				
Serum	**14**	**27.900±0.3211**		**1. p < 0.05***
PBMC	**14**	**15.421±1.7533**	**p = 0.000**	**2. p < 0.05***
Makrofag	**27**	**2.833±0.2717**		**3. p < 0.05***
Endorphin day 21				
Serum	**14**	**7884.179±205.5857**		**1. p < 0.05***
PBMC	**14**	**3219.714±393.0592**	**p = 0.000**	**2. p < 0.05***
Makrofag	**27**	**3468.074±298.4107**		**3. p < 0.05**
Endocannabinoid day 21				
Serum	**14**	**28.071±0.3604**	**p = 0.000**	**1. p < 0.05***
PBMC	**14**	**13.621±3.7785**		**2. p < 0.05***
Makrofag	**27**	**2.622±0.3598**		**3. p < 0.05***

Table-1. Mean, ANOVA and Duncan test of endorphin and endocannabinoid of serum, PBMC and macrophage before Ramadan fasting, day 7 and day 21. Duncan test 1) before Ramadan compare with day 7 or serum compare with PBMC, 2) Before Ramadan compare with day 21 or serum compare with macrophage, 3) day 7 compare with day 21 or PBMC compare with macrophage. (* = Significant).

Serum endocannabinoid before Ramadan (mean ± SD 27.557 ± 0.4345), day 7 (mean ± SD 27.900±0.3211), and day 21 (mean±SD 28.071 ± 0.3604), ANOVA p = 0.003 with Duncan test day 7 and day 21 were significant ($p < 0.05$) compare with before Ramadan and day 7 compare with day 21 not significant ($p > 0.05$). PBMC endocannabinoid before Ramadan (mean±SD 8.643±1.8084), day 7 (mean±SD 15.421±1.7533), and day 21 (mean±SD 13.621±3.7785), ANOVA p = 0.000 with Duncan test day 7 and day 21 were significant ($p < 0.05$), H-7 and day 7 compare with day 21 were not significant ($p > 0.05$). Macrophage endocannabinoid before Ramadan (mean±SD 2.848±0.4154), day 7 (mean±SD 2.833 ± 0.2717), and day 21 (mean±SD 2.622±0.3598), ANOVA p = 0.037 with Duncan test day 7 not significant ($p > 0.05$) compare with before Ramadan and day 21 significant ($p < 0.05$) compare with before Ramadan and day 7.

Endorphin before Ramadan, ANOVA p=0.004, with Duncan test, endorphin of PBMC and macrophage were significant ($p < 0.05$) compare with serum endorphin, and not significant ($p >0.05$) endorphin of PBMC compare with macrophage. Day 7 ANOVA p = 0.004 with Duncan test were significant ($p < 0.05$) each other. Day 21 ANOVA p = 0.000 with Duncan test endorphin of PBMC and macrophage were significant ($p < 0.05$) compare with serum endorphin and not significant ($p > 0.05$) endorphin of PBMC compare with macrophage. Endocannabinoid of serum, PBMC and macrophage before Ramadan, day 7 and day 21 significant difference each other (see table-1).

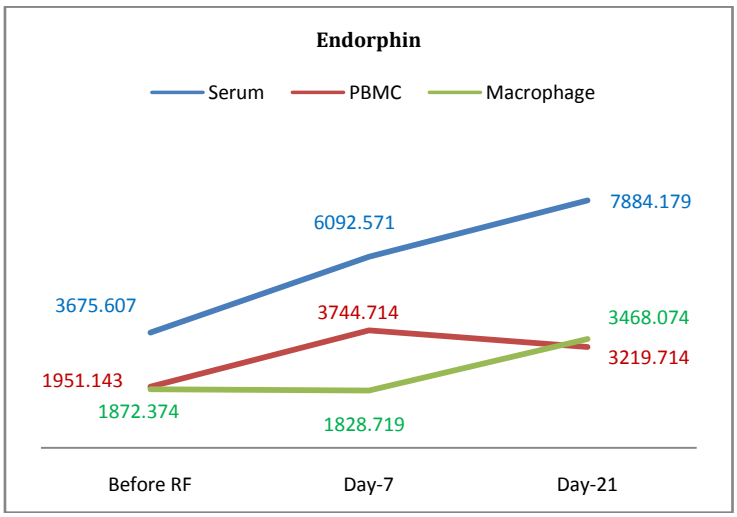

Figure 1. Level endorphin of serum, PBMC and macrophage before RF, day 7 and day 21.

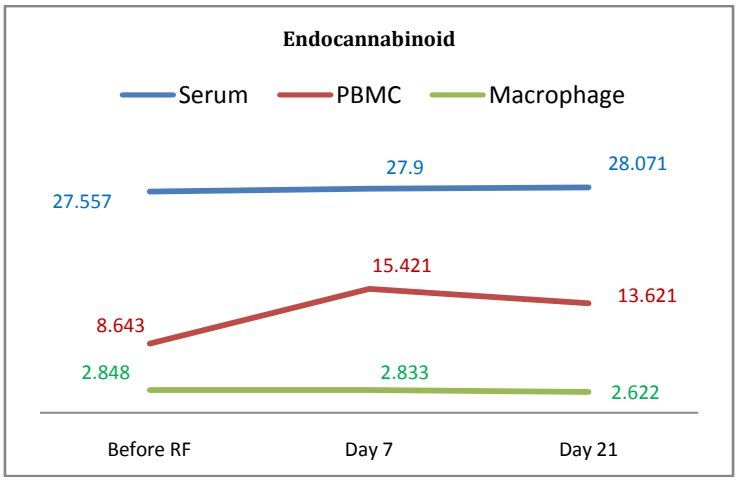

Figure 2. Level endocannabinoid of serum, PBMC and macrophage before RF, day 7 and day 21.

IV. DISCUSSION

Activities during RF (i.e. fasting, praying and wake up in the midnight for sahoor) is one of spirituality practice that done by Muslims in lunar month of Ramadan. Spiritual practices have been proposed to have many beneficial effects as far as mental health is concerned. The role of spirituality as a resource for finding meaning and hope in suffering has also been identified as a key component in the process of psychological recovery.

Most studies have shown that religious involvement and spirituality are associated with better health outcomes, including greater longevity, coping skills and health-related quality of life (even during terminal illness) and less anxiety, depression and suicide. Religiousness is modestly but robustly associated with lower level of depressive symptoms [14].

Religious experience may be a cognitive process, mediated by a pre-established neural circuit, involving dorsolateral, prefrontal, dorsomedial, frontal and medial parietal cortex. There is substantial evidence from the psychology of religion to suggest that people are prepared 'for religious experiences and this readiness' is probably mediated by the dorsomedial frontal cortex, leading to the commonly reported felt immediacy of religious experience. The prefrontal regions mediate both the preparedness of religious experience and conscious cognitive process involved in the appreciation of religious experience [14, 15].

Stress management usually consists of one to all of the following instruments and activities: behavioral or cognitive, exercise, relaxation and nutritional or food interventions (BERN), including social support and spirituality [6]. Spirituality of RF as a stressor will cause stress perception that occurs in a special worship circumstances in which change daily habits. If stress perception against fasting is considered the responsibility, the effect of circadian rhythm will be more dominant [4]. Changes of circadian rhythm will be accepted by hypothalamic suprachiasmatic nucleus [16], that potentially as distress for the body [4,17]. Ramadan fasting if done with sincerity and faith, stress perception emerging to positive coping style (eustress) [4]. The process of spirituality (i.e. meditation, RF) which requires intense focus of attention, seems to activate the PFC (prefrontal cortex) [14]. A balanced function of the medial PFC results in normal error detection, self-reflection and theory of mind resulting in a balanced religious activity [14, 18].

Positive emotional activities have been suggested as modifiers of neuroendocrine hormones involved in the classical stress response. The mirthful laughter experience appears to reduce serum levels of cortisol, dopac, epinephrine, and growth hormone. These biochemical changes have implications for the reversal of the neuroendocrine and classical stress hormone response [19]. Several lines of evidence suggest that the endogenous opioid peptides endorphins may play a role in the defensive response of the organism to stress [20]. In humans, acute stress, which is associated with higher anxiety levels, had increased plasma levels of beta endorphin [21]. Other study showed that beside endorphin, stresses in humans are associated with impaired endocannabinoid activity [22]. Endogenous cannabinoids play an important role in the physiology and behavioral expression of stress responses [23]. Deficits in endocannabinoid signalling may result in depressive and anxiogenic behavioral responses [24].

Our study reveal that serum endorphin (figure-1) and endocannabinoid (figure-2) day 7 and day 21 significantly increase ($p < 0.05$) compare with before RF, but endocannabinoid day 21 not significantly increase ($p > 0.05$) compare with day 7 (table-1). The result showed that all participants were tolerant with RF, in which endorphin and endocannabinoid increase until day 21. These results analogous with study by Zafary & Bakhtiarian (2001) show that plasma beta-endorphin level increase during RF [25]. Plasma beta endorphin is elevated in the early phase of fasting, while not directly being associated with body weight changes. Plasma beta endorphin is lower and less activated in subjects who are able to tolerate fasting for longer periods [26].

Other study by Gustav Schelling reveal that Stress-tolerant participants showed a significant increase in plasma endocannabinoid concentrations, whereas highly stressed individuals showed an absent endocannabinoid response. Physical stress in trained and physically fit individuals induced by hard exercise during mountaineering or cycling also resulted in elevated endocannabinoid concentrations, which returned to baseline after termination of the stressful activity. In contrast, chronically stressed individuals with traumatic memories from war and torture experiences with and without Posttraumatic stress disorder (PTSD) showed persistent elevations of plasma endocannabinoid concentrations when compared to non-traumatized controls. Endocannabinoid plasma levels correlated with scores on the clinician-administered PTSD scale. Analogous findings came from an earlier study in patients with heart disease awaiting cardiac surgery. The patients with traumatic memories and evidence of PTSD from previous life-threatening experiences associated with cardiac disease and evidence of PTSD had significantly higher endocannabinoid plasma concentrations than patients without traumatic memories and PTSD symptoms [27].

Our result also shows that RF as a stressor induces stress response in subjects. A psychological and physiological stress, which stimulates the secretion of endorphin are secreted to counter the negative effects of stress [28]. Beta endorphin is a pro-opio melanocortin (POMC)-derived peptide and is predominantly produced in the pituitary gland and the brain [29]. Beta endorphin has been identified in human plasma [30]. Release of beta endorphin from the anterior pituitary into the general circulation in response to stress and pain is well recognized, but beta endorphin is rapidly degraded by blood proteases [31].

Psychological and physical stress act synergically to increase the levels of beta-endorphin and adrenocorticotropic hormone (ACTH) during the practice of physical exercise [32]. Its effects, such as analgesia, increasing lactate tolerance, and exercise-induced euphoria, are important for training [33]. With placebo, mood states became calmer, more relaxed and pleasant, tending away from depression, anger and

confusion. Positive mood shifts did not occur when subjects were preloaded with naltrexone, suggesting that activity-generated mood changes are mediated through endorphinergic mechanisms [34]. Contras study showed that the increase in beta endorphin can cause uncomfortable feelings (negative mood) that leads to symptoms of depression or distress [35,36,37].

Beta endorphin level decrease in cerebral bleeding [38], alcoholic [39] and cancer pain, in which endorphin level increase again if the pain relief [40]. Beta-endorphin were significantly elevated in patients with major depression [35,36] and postpartum depression [37]. Endorphin can improved with exercise [33,41], and more improve if accompany with loud music [42], but decrease if over training [33].

The central endocannabinoid system is a neuroactive lipid signalling system in the brain which acts to control neurotransmitter release. The expression patterns of this system throughout limbic regions of the brain ideally situate it to exert regulatory control over emotional behavior, mood and stress responsivity. A growing body of evidence unequivocally demonstrates that deficits in endocannabinoid signalling may result in depressive and anxiogenic behavioral [24].

Under the acute stress during parabolic flights, individuals who showed no evidence of motion sickness were in low-stress conditions and had a significant increase of plasma endocannabinoids. In contrast, highly stressed individuals with severe motion sickness had an absent endocannabinoid response and a massive increase in hypothalamic-pituitary-adrenal axis activity. Likewise, chronic but well-tolerated exposure to weightlessness and emotional and environmental stressors on the International Space Station (ISS) for 6 months resulted in a sustained increase in endocannabinoid blood concentrations, which returned to baseline values after the cosmonauts return. Complex environmental stressors result in an increase of circulating endocannabinoid and that enhanced endocannabinoid signaling is probably required for adaptation and tolerance under stressful conditions [43].

Different result shown in PBMC and Macrophage in which PBMC endorphin and endocannabinoid day 7 and day 21 elevated significant compare with before Ramadan ($p < 0.05$), whereas PBMC endorphin day 21 decrease significant compare day 7 but not significant in endocannabinoid ($p > 0.05$). This is shown that response stress of PBMC higher in day 7 significantly and decrease in day 21. Stress response of macrophage shown that macrophage endorphin day 7 decrease not significant ($p > 0.05$) and day 21 increase significant compare before Ramadan ($p < 0.05$). Endocannabinoid day 7 decreases not significant but day 21 decrease significantly compare before Ramadan ($p > 0.05$). Endorphin levels of serum highest both before and after RF compared with PBMC and macrophages, the difference was significant ($p <0.05$). While in the before RF levels of endorphin in PBMC was not significantly higher than in macrophage, but at the beginning of Ramadan the difference become significant and at the end of Ramadan endorphin macrophage be higher than PBMC, although the difference was not significant. Endocannabinoid levels before and after fasting highest in serum and lowest in macrophage with significantly differences (Table-1).

This result showed that RF induces stress response on PBMC and macrophage, in which response stresses of PBMC were in early, while the macrophages were in late RF. Other study reveals that various types of physiological stressors, including physical exercise and emotional stress, can influence immune function [44,45]. Natural immunity is strongly influenced by chronic exercise and this regulation includes interaction between the nervous, endocrine and immune systems. Central mechanisms including the endogenous opioids are of great interest. Chronic activation of endogenous opioid systems augments natural cytotoxicity and the possible involvement the opioids in the exercise-induced enhancement of natural immunity [45].

The present report meta-analyzes more than 300 empirical articles describing a relationship between psychological stress and parameters of the immune system in human participants. Acute stressors (lasting minutes) were associated with potentially adaptive upregulation of some parameters of natural immunity and downregulation of some functions of specific immunity. Brief naturalistic stressors (such as exams) tended to suppress cellular immunity while preserving humoral immunity. Chronic stressors were associated with suppression of both cellular and humoral measures [46]. Chronic stress associated with an inflammatory disease has been shown to increase expression of beta endorphin in immune tissues [31]. Endogenous opioid peptides represent the group of bioregulatory factors possessing a wide range of biologically active effects. One of most essential functions of endogenous opioids appears to be the realization of cellular interaction between nervous and immune systems. Beta endorphin, it's production by the immune system cells, opiate receptor structure and expression, as well as the peptide effect on the processes of cellular activation, proliferation, and differentiation in innate and adaptive immunity [47].

Several study of endorphin effect on immune system showed that beta endorphin induced differentiation of macrophages from bone marrow cells in a semisolid culture system [48], beta endorphin significantly and dose dependently enhanced IFN-gamma and IL-2 in human PBMC [49] and beta endorphin stimulate human neutrophil and mononuclear cell chemotaxis [50]. The enhanced level of beta endorphin causes inhibition of human T helper cell function, which potentially down-regulate the antibody production. Also the beta endorphin-induced enhancement of the natural killer cell activity may suppress the B cell function. In

addition, beta endorphin also exerts a direct inhibitory effect on the antibody production [51], beta endorphin shifts T-helper polarization towards Th-2 cells with subsequent predominance of the humoral form of the immune response [52]. Exogenous beta-endorphin enhances the IL-1 beta, IL-2, IL-8 and iNOS mRNA expression of peripheral blood MNCS [38].

Endocannabinoids are believed to control immune functions and play a role in immune homeostasis [53]. The endocannabinoid system has become a topic of great interest in pharmacology due to its remarkable distribution in mammal organisms and capacity to play a modulatory role on several physiological systems, including modulation of immunity. Many studies have shown that administration of endocannabinoids causes inhibitory effects on immune cells In contrast, other groups have shown that some endocannabinoids might present stimulatory actions on macrophage activity and T cell activation [54], Macrophages play an important role in both innate and adaptive immunity by mediating their effect through presenting antigen to T cells, phagocytosis of infectious agents and secreting acute phase proteins such as nitric oxide, TNF-α, IL-1 and IL-6. CB1 and CB2 receptors are widely expressed on monocytes/macrophages and microgial cells [53,55]. In which IFN-gamma is a mediator of CB2 signaling [56) and endoccannabinoids-induced ROS production by macrophages was CB1-dependent [57]. Reduced macrophage chemotaxis by cannabinoids was observed in a murine model of atherosclerosis, suggesting their strong effect on macrophage migration which is mediated by CB2 receptor signaling [53].

There is significant biochemical evidence to suggest that biosynthesis, uptake and degradation of endocannabinoids occur in macrophages and leukocytes [58,59,60]. This finding supports the role of endocannabinoids as local modulators of immune and inflammatory reactions [53]. There have been a number of recent studies which have demonstrated that the endocannabinoids have both inhibitory effects and stimulatory impact on the immune system and may be actually important in homeostasis or control of the immune reactions [53].

V. CONCLUSION

Our study suggests that RF induce stress response on serum, PBMC and macrophages. While PBMC is higher than macrophage at the beginning of Ramadan and the difference was significant, macrophage higher at the end of Ramadan compared with PBMC although the difference was not significant. But are the serum, PBMC and macrophage during RF in distress or eustress condition and are culture process contribute to stress response of macrophage still unknown

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