

INDONESIAN JOURNAL OF
**Clinical Pathology and
Medical Laboratory**

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

IJCP & ML (Maj. Pat. Klin. Indonesia & Lab. Med.)	Vol. 19	No. 3	Hal. 141-219	Surabaya Juli 2013	ISSN 0854-4263
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Diterbitkan oleh Perhimpunan Dokter Spesialis Patologi Klinik Indonesia

Published by Indonesian Association of Clinical Pathologists

Terakreditasi No: 66b/DIKTI/KEP/2011, Tanggal 9 September 2011

INDONESIAN JOURNAL OF
**CLINICAL PATHOLOGY AND
 MEDICAL LABORATORY**

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Ucapan terima kasih kepada penyunting Vol. 19 No. 3 Juli 2013

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Dewan Redaksi Majalah IJCP

APOPTOSIS INDEX BETWEEN FEMALES AND MALES IN REGULAR HEMODIALYSIS

(Indeks Apoptosis antara Perempuan dan Laki-Laki pada Hemodialisis Reguler)

Djoko Santoso

ABSTRACT

Many reports have documented apoptosis index in hemodialysis patients, but to date, no single study has directly compared the apoptosis index of males to females. Data on mortality rate among hemodialytic patients in the hemodialysis center at the Department of Internal Medicine Dr Soetomo General Hospital, Surabaya, Indonesia show a high number predominated by female patients. Therefore, to answer the question of whether there is a gender difference in apoptosis index, the researcher studied leukocyte responses in male and female hemodialysis patients. The apoptosis index of the sample was measured by indirect immunoassay method. Cell lyses, followed by immunochemical determination of histone-complexed DNA fragments in a microtiter plate wells. The apoptosis quantization was obtained by determining the amount of colored product spectrophotometrically. One hundred and four non-diabetic subjects who received hemodialysis (HD), and 24 normal controls (NC), were evaluated. The apoptosis index in ESRD patients group and control group showed no significant difference (0.6172 vs 0.4008, $p=0.114$), neither did it vary in both sexes and age groups. When the sex factor was analyzed (after exclusion from the diabetic ESRD patients), females apoptosis index was significantly higher than that of the males (0.7325 vs 0.55175, $p<0.05$). In conclusion, apoptosis index in females among non-diabetic patients undergoing hemodialysis is higher than that occur in males and controls.

Key words: Apoptosis index, hemodialysis, gender

ABSTRAK

Banyak laporan telah memuat indeks apoptosis di pasien hemodialisis. Namun sampai saat ini, tidak ada satupun penelitian yang langsung membandingkan indeks apoptosis perempuan terhadap laki-laki. Data angka kematian di antara pasien hemodialisis di pusatnya di Departemen Penyakit Dalam RSUD Dr. Soetomo, Surabaya, Indonesia cukup tinggi dan lebih banyak pengidap perempuan. Oleh karena itu, untuk menentukan perbedaan terkait jenis kelamin dalam indeks apoptosis, diteliti respons leukosit di pasien hemodialisis laki-laki dan perempuan. Untuk mengetahui indeks apoptosis, sampel diukur dengan metode imunoasai indirek. Lisis sel, diikuti dengan penentuan imunokimiawi dari fragmen DNA terhimpun histon dalam piring mikrotiter. Kuantisasi apoptosis dilakukan dengan menentukan jumlah hasilan yang berwarna secara spektrofotometri. Sebanyak 104 subjek non-diabetes yang menjalani hemodialisis (HD) dinilai, dan 24 orang yang sehat (NC) sebagai pembanding. Indeks apoptosis di kelompok pasien ESRD dan kelompok pembanding menunjukkan tidak ada perbedaan yang bermakna (0,6172 vs 0,4008, $p=0,114$). Di samping itu juga tidak beragam di kedua jenis kelamin dan kelompok umur. Ketika faktor jenis kelamin dianalisis (setelah tidak disertakan dari pasien ESRD diabetes), indeks apoptosis perempuan lebih tinggi secara bermakna dibandingkan dengan laki-laki (0,7325 vs 0,55175, $p<0,05$). Berdasarkan telitian ini dapat disimpulkan bahwa di kelompok perempuan non-diabetes yang menjalani hemodialisis, indeks apoptosis lebih tinggi dibandingkan dengan kelompok laki-laki dan pembandingnya.

Kata kunci: Apoptosis indeks, hemodialisis, jenis kelamin

INTRODUCTION

In 2000-2003 a high rate of mortality can be found in the hemodialysis center, 28.4%.¹ Hemodialysis (HD) patients show the characteristic accelerated rate of apoptosis, and this can play a key role in the defective immune response of these patients. This matter could be the progressive worsening of the overall clinical condition.²⁻⁴

Accelerated mononuclear cell apoptosis and high levels of pro-inflammatory cytokines are often seen in patients with uremia.⁵ An increased apoptotic

rate of peripheral blood mononuclear leukocytes in hemodialysis patients has been reported in several studies.^{3,6}

Apoptosis, a physiological suicide mechanism, is the most common form of eukaryotic cell death. This type of cell death naturally occurs during normal tissue turnover, embryonic development of tissue, deletion of autoreactive T-cell and following removal of specific growth factors like interleukin (IL)-2 or addition of physiological stimuli like tumour necrosis factor (TNF) and glucocorticoids.⁸

Department of Internal Medicine, Airlangga University Faculty of Medicine
Dr Soetomo General Hospital, Surabaya, Indonesia. E-mail: drdjokosantoso@yahoo.com

Inappropriate regulation of apoptosis may play an important role in many pathological conditions. Monocyte/macrophage plays a major role of apoptosis in patients suffering from end stage renal disease (ESRD), who generally have immunodeficiency. The existence of a persistent toxin is one of the monocyte stimulations to keep active. Several studies found that the monocytes of patients with chronic renal failure have high levels of scavenger receptors, TNF receptor (which is a marker of the active monocyte) compared to the healthy group. Such condition has also been demonstrated in patients with ESRD who underwent dialysis and those not yet on dialysis.⁹

Toxins continuously exist in patients with terminal renal failure. The toxins are responded by the ability of the body through immune response to minimize biological shock of the cells in their involvement in body reaction against urea. This understanding underlies immunological processes that lead to the protection with two possibilities, mild or severe shock in the form of apoptosis if the problem continues without interruption.¹⁰ This response includes the body's resistance to stressors, in particular in the mechanism of homeostasis. This matter can be understood that monocytes proliferate and /or undergo apoptosis in order to balance the stable condition.

Numerous studies have documented the gender difference in the immune response of healthy adults. However, there is only limited information regarding whether such a difference exists in Chronic Kidney Disease patients. As far as the researcher's knowledge is that only a handful of studies have compared how males and females respond to injury. The researcher's data on hemodialysis patients revealed that gender is a variable to be considered in predicting patient outcome, due to a higher mortality observed in females compared with males receiving hemodialysis in a certain period. Although many reports have documented apoptosis index, but to date, no single study has directly compared the apoptosis index of male to female hemodialysis patients. Therefore, to answer the question of whether there is a gender difference in apoptosis index, leukocyte responses in male and female hemodialysis patients have been studied.

MATERIALS AND METHODS

Among all patients admitted to the Dr Soetomo General Hospital in 2004, 104 subjects of both sexes were eligible for this study. Apotosis was also obtained from 24 healthy controls. Patients with DM, those showing clinical of infection or malignancy, and

those taking immunosuppressive medication known to interfere with the immune system, were excluded from this study.

All the patients had been hemodialyzed for at least 6 months. Bicarbonate dialysate solutions were used, the blood flow was 175–225 mL/min, and dialysate flow rate was 500 mL/min in patients receiving HD. Blood samples were collected before starting the hemodialysis procedure in HD Patients. Antihypertensive drugs were taken when needed. Blood samples were collected in sterile tubes, containing EDTA.

Blood was taken using anticoagulant, centrifuged and then the buffy coat was removed. The white blood cells were lysed with buffer lysate, centrifuged, and the filtrate was analysed by ELISA kit. The method using ELISA kit is an indirect enzyme immunoassay employing mouse monoclonal antibodies for measurement of soluble nuclear matrix protein. Results are determined by a spectrophotometer with an absorbance of 450–595 nm. Minimal detection of the reagent is 10 U/mL.

The Measurement of Apoptotic Cell Death in Cellular Systems

The assay was based on quantitative sandwich-enzyme-immunoassay-principle using mouse monoclonal antibodies directed against DNA and histones, respectively. The sample (cell-lyzate, serum) was placed into a streptavidin-coated MP. A mixture of anti-histone-biotin and anti-DNA-POD was added and incubated. During the incubation period, the anti-histones antibody binded the histones-component of the nucleosomes and simultaneously capture the immunocomplex to the streptavidin-coated MP via its biotinylation. Additionally, the Anti-DNA-POD antibody reacted with the DNA-component of the nucleosomes. The next step was removal of the unbound components (antibodies) by a washing step. The amount of nucleosomes was followed by immunochemical determination of histone-complexed DNA fragments. Apoptosis quantification was obtained by determining the intensity of the colored product spectrophotometrically. One hundred and four non-diabetic subjects who received hemodialysis (HD), and 24 normal control (NC), were evaluated.

Statistical Analysis

The data were expressed as means \pm SD. Statistical analysis was performed using the non parametric ANOVA test to compare the data between these three groups to find the differences between the two groups. Statistical significance was assumed at $P < 0.05$.

Table 1. Baseline characteristics of patients

Characteristics	Patients (n=104)
Age, years old	44.24 (\pm 13.84)
Sex,	
Female	36
Male	68
Patients based on frequency of HD	
HD<2 times a week	61
HD=2 times a week	43
Laboratory data:	
Hb (gr/dL)	7.8 (\pm 1.95)
BUN (mg/dL)	72.6 (\pm 26)
Creatinine (mg/dL)	13.1 (\pm 4.15)
Albumin (g/dL)	3.8 (\pm 0.39)

RESULTS AND DISCUSSION

The apoptosis index in HD patients group and control group showed no significant difference (see Table 2), neither did it vary in both sexes and age groups.

When the sex factor was analyzed, females apoptosis index was significantly higher than that of the males (see Table 3).

The female apoptosis index was also higher than the control group (see Table 4), while males apoptotic index was not significantly different compared to the control group (see Table 5). Not only the frequency of dialysis, but also the age factor was analyzed, yet the females apoptosis index outnumbered the males index.

Apoptosis process is initiated by several numbers of different stimuli such as oxidative stress, cytokine, including uremic syndrome.¹¹ Toxins in CKD (Chronic Kidney Disease) patients responded by macrophages via a complex intracellular mechanism in which eventually it gives rise to a number of active products,

Table 2. Comparison of apoptosis index between control group and patient group

Sample Group	Apoptosis Index (means)	Statistic
HD patients (n=104)	0.6172 \pm 0.6799	Non-parametric ANOVA test
Control (n=24)	0.4008 \pm 0.5453	P=0.114 (NS)

NS, not significant

Table 3. Comparison of apoptotic index between males and females among patients group

Sample Group	Apoptotic Index (means)	Statistic
Female (n=36)	0.7325 \pm 0.7023	Non-parametric ANOVA test
Male (n=68)	0.5517 \pm 0.6182	P<0.05

Table 5. Comparison of apoptotic index between male groups and control group

Sample Group	Apoptotic Index (means)	Statistic
Male		
HD<2 times a week (n=41)	0.6280 \pm 0.6839	Non-parametric ANOVA test
HD 2 times a week (n=27)	0.4863 \pm 0.5524	P>0.05 (NS)
Control (n=5)	0.9800 \pm 0.9697	

NS, not significant

Table 4. Comparison of apoptotic index between female group and control group

Sample Group	Apoptotic Index (means)	Statistic
Female		
HD<2 times a week (n=20)	0.6775 \pm 0.6249	P=0.863(a)
HD 2 times a week (n=16)	0.7875 \pm 0.7797	P=0.007(b)
Control (n=19)	0.2463 \pm 0.2321	P=0.009(c)

(a) = HD<2 times a week compared with HD 2 times a week (not significant)

(b) = HD 2 times a week compared with control

(c) = HD<2 times a week compared with control

such as TNF- α . This apoptotic process is in either undergoing or not yet undergoing hemodialysis.¹²

Previous studies have documented impaired functions of immune cell types in the hemodialysis patients, including macrophages which play a central role in triggering the immune dysfunction.¹³

This macrophage activation becomes more intensive by the increase of lysosomal activation.¹⁴ Consequently, this activated macrophage may secrete cytokines (IL-1, IL-6, TNF- α) that play a role in inducing the inflammatory reaction and induce bone marrow to increase leukocyte production. The occurred leukocytosis is addressed to control apoptotic process.¹⁵

When this pathway does not occur, the predominant one will be the production of TNF- α , and then if TNF- α binds with FAS from cells, apoptosis may occur and becomes difficult to be controlled. This process is confirmed by Kuby who found in his study that cell death mechanism can take place through the binding between ligands and FAS on the cell surface. Furthermore, the presence of L-F binding on cell surface may trigger Fas-associated death domain (FADD) activity, and the FADD activates caspase cascade that induce DNA-ase thus DNA-ase damage DNA resulting in apoptosis.¹¹ In this study the apoptosis index was observed in HD patients with

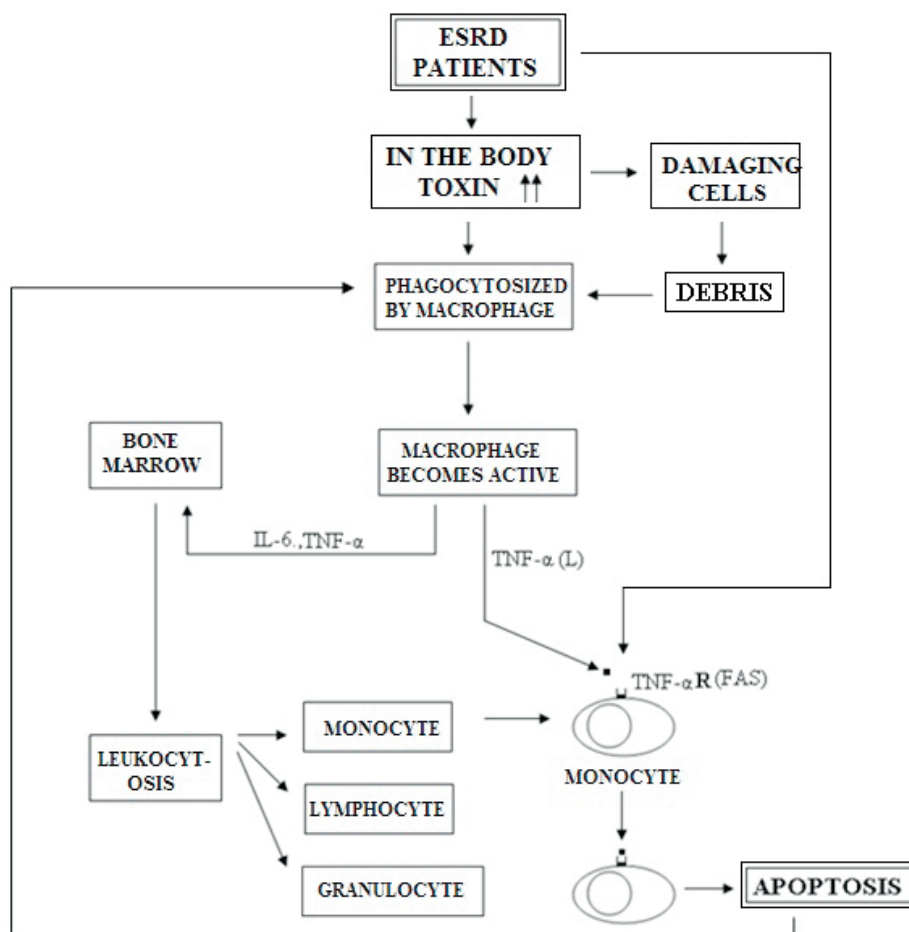


Figure 1. The concept of leukocytes apoptotic balance in patients with ESRD^{2,3,6,7,9}

non-DM underlying disease. The researcher found that the apoptosis index was elevated in the HD patients which suggest that immune function in HD patients is influenced by the uremic toxin.

In this study, based on statistical analysis, it is found that there are differences in the levels of apoptosis index between male and female hemodialysis patients and also in the normal group. In the female group the apoptosis index was significantly higher than that in the male and normal groups. The elevated apoptosis index in females suggests the gender as a prominent influential factor such as the one mentioned in the theory. It is very interesting that according to the facts found in this study the gender factor is worth to consider. However, the researcher was not able to explain why there is a difference in apoptosis index in male and female HD patients. This suggestion is reasonable because of various factors influencing the process of apoptosis, such as dialysis, frequency of hemodialysis, and age.^{16,17}

After statistical tests, these factors were found not to differ significantly between the two groups. Important factors, such as underlying disease of DM, in this study have been controlled through the process of

exclusion. If the researcher refers to the previous study, it has been declared that the presence of antibody and CTL (Cytotoxic T Lymphocyte) are stimulated by exposure to toxic substances that have a close affinity.¹⁸ Phagocyte elements response circulating in CKD body will try to normalize and maintain the homeostasis of apoptosis adverse conditions.¹⁹

The number of antibody products depends on the nature of the cause and also on the characteristics of the hosts, in addition to age, genetic, metabolic, microbial, as well as gender factors,^{8,20} the researcher suggest that the immune function in HD patients is also influenced by levels of gonad steroid hormones. Therefore, the researcher suggest that elevated apoptosis index of mechanism is thought to be related to gonad hormone and activated macrophages which release of TNF- α and IL-6. TNF- α and IL-6 have the ability to trigger an increase in bone marrow tissue leukocytes that are intended to maintain homeostasis.¹¹ Based on this idea, there emerges a notion that apoptosis depends on the levels of IL-6 and TNF- α . If both are within the prime framework, cells undergoing apoptosis and cells produced are in balance.²¹

On the other hand, as suggested by Descamps-Latscha (1996), urea itself also causes tissue injury that will be followed by an increase in apoptosis process, so that the combination of the above will add greater effect to the process.²² If the urea is only temporary, the body response can overcome this. However, if the toxin continues, the production of IL-6 and TNF- α is not balanced so that there will be a lack of IL-6 elements that lead to increased susceptibility to toxic load.²³ In this study, the high apoptotic index in female group may be associated with IL-6 level which, unfortunately, was not measured. Although the researcher did not examine IL-6 in this study, referring to previous studies, it was found that IL-6 level is lower in females than in males in this HD population.²⁴ Thus, it seems reasonable to guess that this difference may be affected by sufficient activity of IL-6. Based on the previous study, in HD patients, toxin enhances IL-6 secretion by toxin-activated human peripheral blood mononuclear cells and macrophages.^{25,26} This is accomplished, in part, by the production of other cytokines, including tumour necrosis factor α (TNF- α).⁹

Chia-Chao also suggests that in ESRD patients, their monocytes may produce pro-inflammatory cytokines, such as IL-1 β , IL-6, TNF- α , and TNF- α -R which in monocytes is also increased.¹⁸ In this study, a high apoptotic index in women may be explained by such a concept. The immune function in HD patients is also influenced by levels of gonad steroid hormones.

Like many other diseases, accelerated apoptosis in Chronic Kidney Disease appears both in females and males.²⁷ Due to the differences of hormones, apoptosis index in females differ from that in males. In contrast, even though our study exhibits a significant difference in gender, but the results of our study do not support the idea that apoptosis in male patients with chronic renal diseases was significantly higher compared with female subjects.²⁸ Then, what are the specific causes for females to get this? First of all, compared with males, females have a poor immunity, which makes females prone to get diseases. Moreover, due to the poor immunity, females are easier infected than males. In addition, infection is the inducing factor of apoptosis. The host immune mechanisms that can be viewed as a whole as a set of protective instruments comprising various components that protect the host from adverse effects of harmful stimulators. In CKD conditions, which is generally aggravated by malnutrition, defects may be found in all aspects of body defences, at least phagocyte dysfunction.^{5,8} It is not surprising that the nutritional given factor was evident in studies of malnutrition in developing countries and highly correlated with failure of development of immune response in young people with nutritional deficiencies. Although this study did not directly observe the

measurement of malnutrition parameter, earlier study from the same population demonstrated that the presence of malnutrition of over 60 percent are very likely to aggravate the process of apoptosis.²⁹

CONCLUSIONS

This study report the demonstration that there are differences in apoptosis index of male and female hemodialysis patients. The apoptosis index in females among non-diabetic patients undergoing hemodialysis is higher than that in males and controls. Further studies must be performed to fully understand the mechanisms which may regulate this difference and to confirm the result of this research.

ACKNOWLEDGMENT

The author acknowledges Dr. I Ketut Sudiana, M.Kes for his suggestions and support in the preparation of this article.

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