J Med Sci
Volume 43, No. 2, June 2011: 79-86

A deterministic and stochastic approach to analyze carbon tetrachloride-induced liver injury in rats

Ngadikun*
Department of Biochemistry, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

ABSTRACT

A new spectrometrical method was developed to determine a marker for liver damage. The aims of this study was to investigate the pattern of cellular interaction in EDTA-blood spectrometrically in rats that induced by carbon tetrachloride (CCl_4). Eight of white male rats (*Rattus norvegicus* L), aged of 2 months with body weight of 160-210 g were divided into two groups with 4 rats in each group. The first group as control were not fed and the second group as treatment were fed with 0.1 mL/kgBW CCl_4 for 21 weeks. At the end of the experiment, rats were fasted overnight, 3.0 mL of blood was drawn from the vena orbitalis for spectrometrical and biochemical estimation. Rats were then sacrificed and the liver tissue was used for histological assessment. All data were analyzed with t-test. Histopathological studies of treated group showed the damage of the liver cells compared with control group. The results showed no significant difference in ALT ($p = 0.12$) and AST ($p = 0.19$) but significant difference in AST/ALT ratio ($p = 0.01$) between 2 groups was observed. The deterministic *and stochastic approaches* showed no significant differences in k7 ($p = 0.11$) but significant difference in k6 ($p = 0.00$) and k8 ($p = 0.00$). It was concluded that the combination of deterministic and stochastic approaches can be used to determine a marker for liver damage.

Key words **:** CCl_4*-induced liver injury* – dendrogram – deterministic - *stochastic approach* - non-functional plasma enzymes - *Rattus norvegicus*.

ABSTRAK

Metode spektrometri baru telah dikembangkan untuk penentuan marker adanya kerusakan sel hati. Tujuan penelitian adalah untuk menyelidiki pola interaksi selular dalam darah-EDTA secara spektrometri pada tikus yang diinduksi dengan CCl_4. Delapan ekor tikus putih jantan (*Rattus norvegicus*), berumur 2,0 bulan dengan berat badan 160-210 g dibagi 2 kelompok dengan 4 ekor masing-masing kelompok. Kelompok I sebagai kelompok kontrol tidak mendapat perlakuan. Kelompok II sebagai kelompok perlakuan diberi CCl_4 dosis 0,1 mL/kgBW selama 21 minggu. Pada akhir percobaan, tikus dipuasakan. Sebanyak 3,0 mL darah diambil dari vena orbitalis untuk pemeriksaan spektrometri dan biokimia. Selanjutnya tikus dikorbankan dan diambil organ hatinya untuk pemeriksaan histologi. Perbedaan dua kelompok dianalisis menggunakan uji-t. Dari pemeriksaan histopatologi dijumpai adanya kerusakan hati pada kelompok perlakuan, yang tidak dijumpai pada kelompok kontrol. Hasil penelitian menunjukkan tidak terdapat perbedaan bermakna untuk AST dan ALT (masing-masing $p = 0,12$ dan 0,19) pada kedua kelompok, namun terdapat perbedaan bermakna untuk rasio AST/ALT ($p = 0,01$). Pada pendekatan deterministik dan stokastik diperoleh hasil tidak berbeda bermakna untuk k7 ($p = 0,11$), namun terdapat perbedaan bermakna untuk k6 dan k8 (masing-masing $p = 0,00$ dan 0,00). Dapat ditarik kesimpulan bahwa pendekatan gabungan deterministik dan stokastik dapat digunakan sebagai marker adanya kerusakan sel hati tikus yang diinduksi dengan CCl_4.

Kata kunci **:** kerusakan hati akibta CCl_4 – dendrogram – pendekatan deterministik – pendekatan *stochastik* - *enzim plasma* non-fungsional - *Rattus norvegicus*.

* corresponding author: ngadikunugm@yahoo.co.id

INTRODUCTION

Liver disorders are one of the most important world health problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently inadequate. Therefore, so far not yet any therapy has successfully prevented the progression of hepatic disease. Liver injury due to chemical or infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure.[1]

Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in animal model. This model has been used in various studies to evaluate the deposition of extracellular matrix in the fibrotic and cirrhotic liver. Carbon tetrachloride is a selective hepatotoxic chemical agent. Carbon tetrachloride-induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation.[1] A number of investigators have utilized this chemical to produce liver cirrhosis in animal model.[2]

The liver is actively related with the production of plasma proteins. There is a reserve store of protein material in the liver and some of this or other material are released into the circulation after a rapid and extreme plasma depletion to increase the total plasma protein concentration. Thereby, the level of plasma proteins is associated with liver damage by CCl_4.[3]

When anticoagulated blood is allowed to stand quietly, red blood cells adhere together, a process called rouleaux formation, and settle toward the bottom of the container because the density of the cells is greater than that of the plasma. The sedimentation rate of red blood cells is called the erythrocyte sedimentation rate (ESR). The ESR depends on many factors such as the level of plasma proteins that increases rouleaux formation and the subsequent surface-to-volume ratio that favors erythrocyte sedimentation.[4,5]

The ESR can be measured in different ways. As the red blood cells settle, yellowish plasma appears from the upper region of the blood column. It is possible to measure the distance from the meniscus to the top level of the red blood column in a given period of time. Another method is to measure the time at which the top level of the red blood column reaches a designated level. Measuring the sedimentation rate of red blood cells is considered as the most reliable method of obtaining ESR.[6] Spectrometric method can be used to measure ESR by calculating the water content of biological tissues in the upper region of the blood column in time series data.[7]

The signal time series can be decomposed into its deterministic and stochastic components, which are statistically uncorrelated. In a prediction setting, the deterministic portion of the signal is degraded due to the presence of the stochastic component. The portion of the predictable variance equals the variance of the deterministic component. In this report, the deterministic component refers to the component of the signal that can be predicted from a number of previous time samples, whereas the stochastic component refers to the component for which such prediction is impossible.

This study aimed to investigate the time series data of the ESR by deterministic and stochastic approaches to analyze CCl_4-induced liver injury in rats.

MATERIALS AND METHODS

The chemical hepatocarcinogen use in this experiment was CCl_4. Water and basal feed were given ad libitum. Basal feed was produced by PT Japfa Comfeed Indonesia and contained: maximal water (12%), minimal crude protein (19%), minimal crude fat (4%), maximal crude fiber (5%), maximal crude dust (6.5%), calcium (0.9-1.1%), and phosphor (0.7-0.9%).

Animals and surgery

In this study, 8 adult male *Rattus norvegicus* rats, (2 months old) from Animal Laboratory Development Unit, Gadjah Mada University were used. The rats were divided into two groups. In group I (n = 4), no-treatment was given (control); in group II (n = 4), rats were fed with 0.1 ml/ kgBW CCl_4 administered by gavage per os 5 days a week for 21 weeks. Body weight was determined per week.

At the end of the experiment, the animals were fasted overnight, and then 3.0 ml of blood was drawn from the orbital vein of the rats and collected in EDTA-tube and then the rats were sacrificed. Liver and body weight were determined for each group.

Histology

The fixed specimens were dehydrated, cleared, and embedded in paraffin. The serial 6 sections, which were 5 μm thick, were taken from these blocks by classic rotary microtome. All the slides were evaluated under light microscope (a BX51TF Olympus optical microscope).

Laboratory Assays

Plasma was used to measure total protein (TP), non-functional plasma enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (gGT). The measurement was performed using biochemical test kits in SYNCHRON CX® System(s) (Chemistry Information Sheet 389775 AD and 389745 AD).

Blood spectrum study

About 1 ml of EDTA blood sample was placed into a test tube (cuvette) in spectrometer IEG (ISITEKS Electronic Group). The cuvette (10 mm ID, 75 mm long) was designed so that the spectrum of blood could be measured in the upper region of the blood column. The LDR (Light dependent resistor, 3 mm ID) detector converts the intensity of spectrum that across the blood sample in cuvette into an electrical signal, which is recorded by a computer. The spectrum measurements were made every 2 second for 200 discrete data as a time series that automatically saved in a file in hard disk. The time series data were analyzed using MATLAB programs for Windows XP to get deterministic and stochastic parameter. In deterministic model, every set of variable states is uniquely determined by parameters in the model and by sets of previous states of these variables. Conversely, in a stochastic model, randomness is present, and variable states are not described by unique values, but rather by probability distributions.

Protocol of this study has been approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Gadjah Mada University, Yogyakarta.

Statistical analysis

All data were analyzed using t-test of Matrix Laboratory (MATLAB) programs for Windows XP. A p value of less than 0.05 was accepted as statistically significant. The dendrogram was used as a tool for the selecting representative samples for in-depth analysis.

RESULTS

Histopathological study

Histopathological study of the liver section of control and treatment groups are shown in FIGURE 1. The liver section of Group I (control group) had normal architecture, where the central veins, portal tracts, hepatocytes and sinusoids appeared normal. The lobular unit was also well identified. Meanwhile, the liver of Group II (treatment group) was damage. There were various degrees of degeneration, medium-sized nucleoli with rough chromatin, individual hepatocyte necrosis, but unclear hepatic dysplasia. There were also enlargement of portal tracts and central veins, medium polymorphic level, and relatively mild hepatic steatosis.

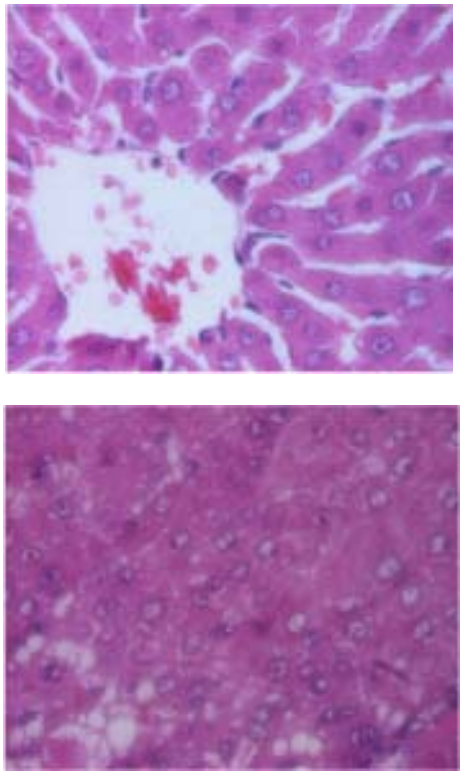

FIGURE 1. A: Control group showed normal hepatocytes. B: treated group (was given CCl_4, administered by gavage,5 days per week for 21 weeks) showed sharp boundaries between the necrotic and normal cells. Original magnification of all panels, X400. Hematoxylin eosin stain.

Physico-biochemical aspects

According to the results of t-test, significant difference was observed between the treated and the control groups in body weight (p = 0.0167), liver weight (p = 0.0249) and AST/ALT ratio (p = 0.0145), but no significant difference was observed in liver/ body weight ratio (p = 0.0575), TP (p = 0.5072), gGT (p = 0.2447), ALT (p = 0.1225), and AST (p = 0.1859) (TABLE 1). Body weight, liver weight, and the liver/body weight ratio of the treated group were higher than those of the control group.

Varied factors might involve in the enlargement of the liver, and up to this time only a few are known. The increase in the percentage of water, as found by McEwen and Haven, although only in limited amounts, seems to be important. It suggests the possibility of systemic changes in the cytoplasm. Yeakel's finding that the increased liver size induced by hypertrophy can increase protein anabolism still has to be proved. Histological investigations indicated that the increased weight of the liver may be explained in part by the mitotic activity of the liver.[9]

TABLE 1. The physic-biochemical parameter of normal and treated rats

Physico-biochemical parameter	Group								
	I (n = 4)				II (n = 4)				
	X	Range		SD	X	Range		SD	p
Body Weight (g)	189.1	162.4	208.2	21.7	288.4	272.1	319.2	21	0.0167
Liver Weight (g)	4.5	4	5	0.5	9.2	7.9	11.9	1.9	0.0249
Liver/Body weight	0.024	0.02	0.027	0.003	0.032	0.028	0.037	0.004	0.0575
TP (g/dL)	6.1	5.7	6.3	0.3	5.8	5.4	6.4	0.45	0.5072
γGT (IU/L)	1.95	0.3	2.9	1.18	5.78	1.5	12.7	4.85	0.2447
ALT (IU/L)	59.1	50.7	74.3	10.4	547.9	208.3	1205.1	454.1	0.1225
AST (IU/L)	110	85.8	126.9	17.4	222.7	33.7	357.7	145.5	0.1859
AST/ALT	1.891	1.556	2.242	0.381	0.5	0.118	0.893	0.37	0.0145

TP: plasma total protein. gGT: gamma glutamyl transferase. AST: aspartate aminotransferase. ALT: alanine aminotransferase. AST/ ALT ratio. I: Normal Group, II: Treatment Group

Deterministic and stochastic study in time series data

A time series was calculated for different simulated suspensions settling, with the diameter range of the column is from 30 nm to 30 ìm. For 3.77 μm particles (about the size of a RBC) in plasma at room temperature, a calculated time series is presented in FIGURE 2.

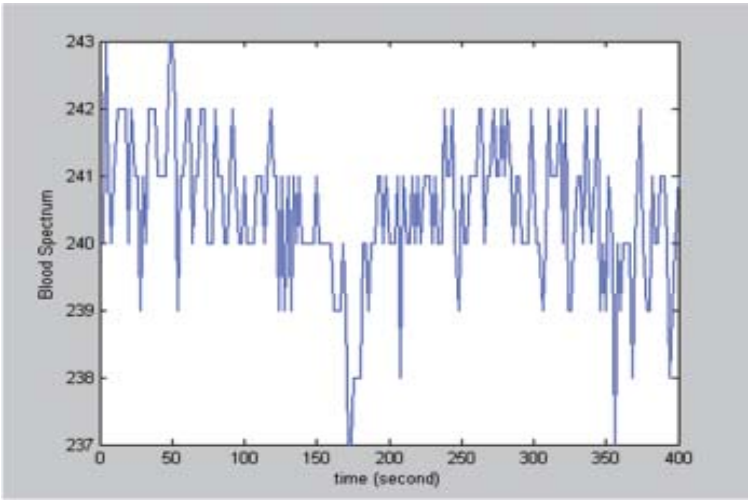

FIGURE 2. A computed time series for 200 discrete data of EDTA blood spectrum: measurements were made every 2 second. The numbers near each line represented ESR measured by the Westergren method

The parameter values of deterministic approach by of linear curve fitting were k1 and k2, while the parameter values of stochastic approach by quadratic curve fitting were k3, k4 and k5. The representative parameter value of deterministic (k6) was ordinate value of linear curve at optimal autocorrelation value, while the representative parameter value of stochastic (k7) was the optimal autocorrelation value. The ideal parameter value (k8) obtained from combining k6 dan k7 was based on Pythagoras' theorem. According to the results of t-test, significant difference was observed between the treated group and the control group in k1 ($p = 0.0177$), k2 ($p = 0.0017$), k6 ($p = 0.0014$), and k8 ($p = 0.0011$); but no significant difference was observed in k3 ($p = 0.4614$), k4 ($p = 0.3095$), k5 ($p = 0.1628$), and k7 ($p = 0.1128$) (TABLE 2).

TABLE 2. Deterministic parameter (k1 and k2), stochastic parameter (k3, k4, and k5), representative deterministic and stochastic parameter (k6 and k7), and ideal parameter (k8) of blood spectrum

deterministic and stochastic parameter	Group								p
	I (n = 4)				II (n = 4)				
	X	Range		SD	X	Range		SD	
k1	-0.036	-0.124	-0.002	0.059	-0.129	-0.218	-0.054	0.068	0.0177
k2	242	237	249	5	196	191	199	3.6	0.0017
k3	0.000028	0.000017	0.000052	0.000017	0.000036	0.000018	0.000051	0.000015	0.4614
k4	-0.00655	-0.01268	-0.00209	0.0046	-0.00989	-0.01244	-0.00672	0.0025	0.3095
k5	0.0183	-0.0165	0.0862	0.0474	-0.0249	-0.0824	0.019	0.0425	0.1628
k6	238	222	248	11	179	172	186	6.4	0.0014
k7	39	8	78	30.2	72	64	78	6.9	0.1128
k8	243	236	249	5.4	193	188	198	5.2	0.0011

k1, k2: coefficients of linear curve fitting from deterministic approach *estimated by linear equation; k3, k4 and k5: coefficients of quadratic curve fitting from stochastic* approach *estimated by autocorrelation function; k6: the representative value of deterministic parameter which is the ordinate value of linear curve at optimal autocorrelation value; k7: the representative value of stochastic parameter which is the optimal autocorrelation value; k8: the ideal parameter value obtained from combining k6 dan k7 based on Pythagoras' theorem.*

The comparison of the level of non-functional plasma enzymes (ALT and AST) and the deterministic and stochastic approaches of time series data (representative parameter of deterministic and stochastic value (k6 and k7) between control and treatment groups is shown in FIGURE 3. There was no significant difference between the two groups for ALT, AST, and k7 ($p > 0.05$), but significant difference was observed for k6 ($p < 0.05$). The comparison of AST/ALT ratio and the ideal parameter of blood spectrum (k8) are presented in TABLE 3. There was significant difference in AST/ALT ratio and k8 between the two groups ($p < 0.05$).

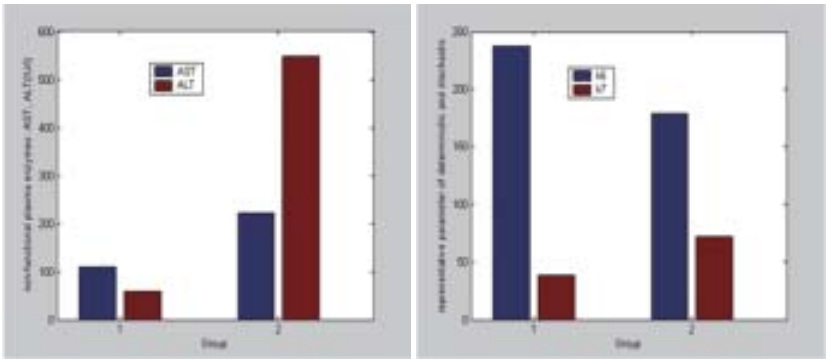

FIGURE 3. A. Histogram of the level of non-functional plasma enzymes (ALT and AST). B. Histogram of the parameter of deterministic and stochastic (representative deterministic and stochastic parameters, k6 and k7). Group I: control group, no-treatment. Group II: treatment group, rats were fed with 0.1 ml/ kgBW of CCl_4 by gavage per os 5 days a week for 21 weeks (n = 4 per group).

TABLE 3. Aspartate aminotransferase to alanine aminotransferase ratio (AST/ ALT) and the ideal parameter of blood spectrum (k8)

Group				Group			
I		II		I		II	
Subject	AST/ALT	Subject	AST/ALT	Subject	k8	Subject	k8
1	1.556	5	0.893	1	241	5	198
2	2.242	6	0.260	2	244	6	197
3	2.199	7	0.727	3	249	7	188
4	1.566	8	0.118	4	236	8	189

Cluster analysis of AST to ALT ratio (AST/ ALT) and the ideal parameter of blood spectrum (k8) are presented in a dendrogram (FIGURE 4) which lists all samples and indicates the level of similarity of two joined clusters. The ordinate (y) represented similarity of parameter value, in which the different values of the measured parameter. [20] The dendrogram in Figure 4 A showed that sample 1 and 4 were the most similar (y= 0.010) and joined to form the first cluster, followed by samples 2 and 3 (y = 0.043). The last two clusters formed was 1-4-2-3 and 5-7-6-8 (the difference of the two clusters was about 0.6). The control group (sample 1 - 4) was in the first cluster, while the treatment group (sample 4 – 8) was in the second cluster. In Figure 4 B, sample 5 and 6 were the most similar (y= 1.00) and joined to form the first cluster, followed by samples 7 and 8 (y = 1.00). The last two clusters formed was 5-6-7-8 and 1-2-3-4 (the difference of the two clusters was about 50).

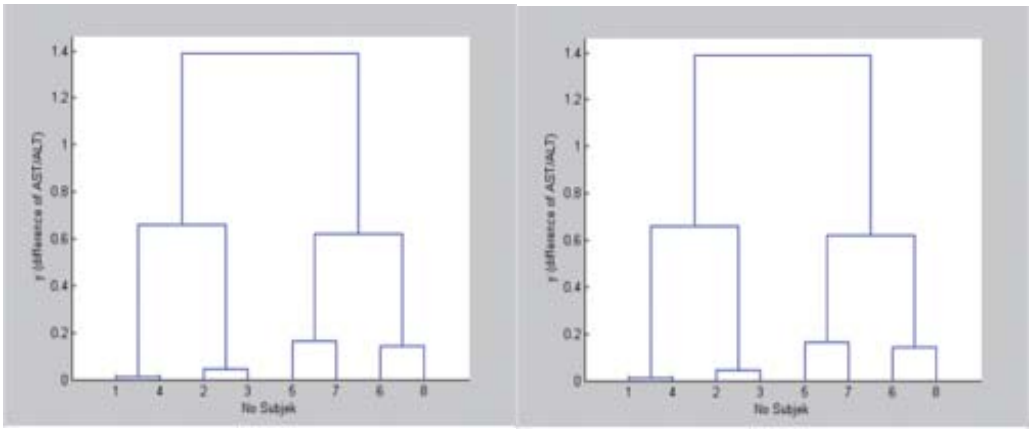

FIGURE 4. A. Dendrogram of AST to ALT ratio (AST/ALT). B. Dendrogram of the ideal parameter of blood spectrum (k8). Group I: control group, no-treatment (sample 1-4). Group II: treatment group, rats were fed with 0.1 ml/ kgBW of CCl_4 by gavage per os, 5 days a week for 21 weeks (sample 5-8).

DISCUSSION

Carbon tetrachloride induces hepatic damage by lipid peroxidation and decreasing activities of antioxidant enzymes and generation of free radicals. Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. Carbon tetrachloride-induced hepatic fibrosis is a well-established animal model to study the pathogenesis and therapy of chronic liver injury.[1]

In this study increased variance in hepatocyte size was observed in livers from CCl_4-treated animals, suggesting cellular proliferation.[8] Total protein in the treated group (5.8±0.45 g/dl) was lower than the control group (6.1 ± 0.26 g/dL). Protein

turnover generally decreases along with the advanced liver disease, most so in patients with hepatic coma where protein synthesis is only one third to one half of that observed in normal individuals.[10] Liver is considered to be the major source of plasma proteins and, damage to this organ leads to the decrease of total protein.[11-13]

Gamma glutamyl transferase in the treated group (5.78 ± 4.85 IU/L) was higher than the control groups (1.95 ± 1.18 IU/L). Dietary steatohepatitis induced by administration of hepatocarcinogen was associated with the increased activity of gGT. Abnormal high activity of gGT appears to be specific for diseases of liver, biliary tract, and pancreas. It is an oncofetal protein, a glycoprotein whose level is altered during development and carcinogenesis. Interest in gGT has focused on its value in the diagnosis of various liver diseases. The severity of fatty liver can be evaluated using gGT values as markers of serious hepatic dysfunction or damage.[14] Alanine aminotransferase in the treated group (547.9 ±454.12 IU/L) was higher than the control group (59.1 ±10.43 IU/L), likewise for aspartate aminotransferase (109.9 ±17.37 IU/L vs 222.7 ±145.46 IU/L). Activities of ALT and AST have been widely used as sensitive laboratory parameters in clinical practice to evaluate the degree of liver injury. Alanine aminotransferase is an enzyme presents in hepatocytes (liver cells), and it leaks into the blood when liver cells are damaged. Alanine aminotransferase level rises dramatically in acute liver damage (such as viral hepatitis and paracetamol overdose) and during liver inflammation. Aspartate aminotransferase is similar to ALT in which it is another enzyme associated with liver parenchymal cells. It increases in acute liver damage and also presents in red blood cells and cardiac muscle. The serum values of these enzymes do not correctly reflect the degree of hepatic cell necrosis. Elevated activity of AST and ALT might be observed when cells containing these enzymes are injured or the permeability of cell membranes increases. Although serum levels of both ALT and AST are elevated when liver cells are injured, the degree of elevation is not parallel to the degree of injury.[15] The mechanism of the elevation is affected by many factors, such as etiology of the liver disease or severity of the liver cell necrosis. The ratio of AST to ALT is a useful parameter which can predict the severity of liver disease.[16]

Deterministic and stochastic study *in* time series data

As the red blood cells settles, plasma appeares in the top region of the blood column. During the aggregation period, red blood cells adhere together and form macromolecules (rouleaux). Then the cells start to settle and soon reach a constant sedimentation rate. The red blood cells maintain the same rate unless the packing effects retard free sedimentation. Erythtocyte sedimentation rates measured by the Westergren method can be measured by the distance travelled by the settling cells over 60 min. The resistance of the lower limit of plasma region settled through the LDR is not constant, instead, it varies according to the light's intensity impacted on it.[6]

In practice, a given time series is not simply deterministic or stochastic, but rather some combination of both. In this study, the deterministic component is estimated by linear equation,[17] whereas the stochastic component is estimated by autocorrelation functions.[18] The goal of data (or curve) fitting is to find the parameter values that most closely match the data. [19]

CONCLUSION

Combining deterministic and stochastic approaches can be used to determine a marker for liver damage by analyzing the pattern of cellular interaction in EDTA-blood spectrometrically of rats that induced by CCl_4.

ACKNOWLEDGMENT

The author would like to thank Dr. Djaka Sasmita for his support in the spectrometer IEG and dr. Totok Utoro, SpPA(K), DSc. from the Department of Anatomic Pathology, Faculty of Medicine, Gadjah Mada University, Yogyakarta for his support in interpreting the histological assessment. Thank also to Prof. Adhi Susanto, MSc., PhD. from the Department of Electrical Engineering Faculty of Engineering, Gadjah Mada University, Yogyakarta for his support in interpreting the signal analysis of time series data.

REFERENCES

1. Kumar PV, Sivaraj A, Elumalai EK, Kumar BS. Carbon tetrachloride-induced hepatotoxicity in rats - protective role of aqueous leaf extracts of coccinia grandis. Int. J Pharm Tech Res 2009;(1):1612-5.
2. Kanter M, Coskun O, Budancamanak M. Hepato-protective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J Gastroenterol 2005;(42):6684-8.
3. Rajan AV, Shanmugavalli N, Greety Sunitha C, Umashankar V. Hepatoprotective effects of *Cassia tora* on CCl_4 induced liver damage in albino rats. Indian J Sci Technol 2009;(2):41-4.
4. Ballou SP, Kushner I. Laboratory evaluation of inflammation. In: Kelley WN, Harris ED Jr, Ruddy S, Sledge CB, editors. Textbook of rheumatology. 4th ed. Philadelphia: W.B. Saunders Co., 1993.
5. Brahn E, Scoville CD. Biochemical markers of disease activity. In: Bird H, Dixon JS, editors. Biochemical aspects of rheumatic diseases. Vol. 2. London: Bailliere Tindall; 1988.
6. Cha K, Brown EF, Wilmore DW. A new bioelectrical impedance method for measurement of the erythrocyte sedimentation rate. Physiol Meas 1994;(15):499-508.
7. Ngadikun. Pengukuran Laju Endap Darah (Erythrocyte Sedimentation Rate) dengan Metode Spektrometri, Tesis S2 UI. Jakarta: FK UI, 1998.
8. Furness PN. The use of digital images in pathology. J Pathol 1997;253–63.
9. Annaua E, Manginelli, Roth A. Increased weight and mitotic activity in the liver of tumor-bearing rats and mice. Cancer Res 1951; 304-6.
10. Matkowitz R, Hartig W, Junghans P, Slowig M, Bornhak H, Faust H, *et al.* Protein turnover in liver failure - determination using the stable isotope 15N [German]. Leber Magen Darm 1984;14:83-9.
11. Egbal S A, Samia AB, Iman ME, and Inaam ELD. Serobiochemical changes associated with highly pathogenic avian influenza in chickens. Res J Animal Vet Sci 2007;(2): 53-5.
12. Somi MH, Rahimi AO, Moshrefi B, Rezaeifar P, Maghami JG. Nutritional status and blood trace elements in cirrhotic patients. Hepatitis Monthly 2007; 7(1): 27-32.
13. Meshkibaf MH, Ebrahimi A, Ghodsi R, Ahmadi A. Chronic effects of lamotrigine on liver function in adult male rats. Indian J Clin Biochem 2006;21(1):161-4.
14. Atakisi E, Karapehlivan M, Atakisi O, Kontas T, Marasli S. Adenosine deaminase and biochemical liver function tests in the dermatophytic cattle. Bull Vet Inst Pulawy 2006;(50):481-3.
15. Tameda M, Shiraki K, Ooi K, Takase K, Kosaka Y, Nobori T, *et al.* Aspartate aminotransferase-immunoglobulin complexes in patients with chronic liver disease. World J Gastroenterol 2005;11(10):1529-31.
16. Powell LW, Bassett MK, Halliday JW: Hemochromatosis: 1980 update. Gastroenterology 1980;78:374-81.
17. Sarkar TK, Nagaraja S,Wicks MC. A deterministic direct data domain approach to signal estimation utilizing nonuniform and uniform 2-D arrays. Digital Signal Processing 1998; 8:114–25.
18. Durgin GD. Theory of stochastic local area channel modeling for wireless communications [Dissertation]. Virginia, Virginia Polytechnic Institute and State University, 2000.
19. Brown AJ. Spectral curve fitting for automatic hyperspectral data analysis. IEEE Trans Geosci Rem Sens 2006;(44):1601-8.
20. Bçrçczkya K, Laatschb H, Wagner-Dçblerc I, Stritzkea K, and Schulz S. Cluster analysis as selection and dereplication tool for the identification of new natural compounds from large sample sets. Chemistry & Biodiversity. 2006 (3): 622-634.