The Effect of Heparmin® on Interferon Gamma Levels and Liver Function in Drug Resistance Tuberculosis Patients

Achmad Furqon[1], Tutik Kusmiati[2], Ulfa Kholili[3]

[1]Resident, [2]Lecturer, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, [3]Lecturer, Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Objective: This study was conducted to determine the benefits of giving Heparmin® containing Kleinhovia hospita, Curcuma xanthorizza, Nigella sativa, Ophiocephalus striatus, and Andrographis paniculata, to drug resistance tuberculosis patients which aims to minimize the side effects of drug induced liver injury (DILI).

Method: This study was a pre-experimental study with a Pre and Post Test Group Design Drug resistance tuberculosis patient treated with a STR regimen plus Heparmin® to determine IFN-γ levels and liver function (aspartate aminotransferase/AST, alanine transaminase/ALT, direct and total bilirubin) before and after treatment at Dr. Soetomo General Academic Hospital, Surabaya and Mohammad Noer Hospital, Pamekasan, Indonesia who met the inclusion and exclusion criteria from February 2020 to February 2021.

Result: This study involved 14 drug resistance tuberculosis patients, the most sex was male 10 patients and 4 female patients, mean age 36.5 years, high school education, normal BMI, GeneXpert mycobacterium tuberculosis Detected Medium, without comorbid with primary cases and re-treatment cases had a comparison the same one. There was sputum culture conversion in all subjects in the second month. The mean value of IFN-γ levels before administration of Heparmin® was 90.61±9.11 ng/ml and after administration of Heparmin® 91.11±8.60 ng/ml. The values of AST, ALT, direct and total bilirubin after administration of Heparmin® are still within normal limits. The results of the paired t test and the Wilcoxon test between before and after giving Heparmin® were not significant.

Conclusion: There was no difference in IFN-γ levels and liver function before and after giving Heparmin® for 2 months.

***Keyword**: IFN-γ, Liver function, drug resistance tuberculosis, Heparmin®*

Introduction

Tuberculosis (TB) is becoming a public health crisis worldwide and globally an estimated 10 million TB cases are reported with 1.2 million deaths in 2019. Indonesia is one of 30 countries with a high burden of TB in the world and is ranked 2nd in the world with 845,000 TB cases, while the drug resistance tuberculosis case was ranked 5th with 24,000 cases in 2019(1). In 2019 in Indonesia around 11,500 drug resistance tuberculosis patients were found and reported, 48% started treatment with a treatment success rate of 45%. 72.4% of patients receiving the drug resistance tuberculosis treatment regimen experienced at least one drug side effect. >90% of patients undergoing drug resistance tuberculosis

Corresponding Author:
Tutik Kusmiati
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: tutik.kusmiati93@gmail.com

therapy experience one drug side effect so that the lost to follow-up rate is high and is a factor in the failure of drug resistance tuberculosis treatment, this is because the number of drugs taken is more, and drug side effects are mild up to weight often occurs. Side effects caused by drug resistance treatment for patient tuberculosis include: hepatotoxic, hypokalemia, hyperuresemia, nephrotoxicity, gatrointestinal disorders, heart disorders, atralgia, ototoxic, optic neuritis, peripheral neuropathy, vertigo, sleep disturbances, depression, skin discoloration and hypothyroidism[2].

Types of anti-tuberculosis drugs that often cause liver function disorders that use a shorter therapy regiment (STR) guide on tuberculosis drug resistance for patients include Pyrazinamide (Z), high-dose Isoniazid (H^{DT}), Etionamid (Eto)/Protionamid (Pto) and Moxifloxacin (Mfx)[2]. Drug induced liver injury (DILI) is liver damage caused by drugs, herbs or chemicals resulting in liver disorders (characterized by abnormal liver function tests) after elimination of several other causes[3, 4]. The incidence of DILI associated with the administration of anti-tuberculosis drugs is reported to be 5-28% with a mortality rate of 4-12%[5].

Interferon gamma (IFN-γ) is a key cytokine for protective immune response against Mycobacterium tuberculosis because it increases the effect of T lymphocytes on alveolar macrophages and stimulates TNF-α secretion by macrophage cells. Patients with pulmonary tuberculosis there was a statistically significant decrease in IFN-γ. Several studies have also shown that there is a decrease in IFN-γ levels in active TB patients. Other studies have found that IFN-γ production is very low in patients with severe TB and suffering from malnutrition. This suggests that the initial immune response to Mycobacterium tuberculosis is associated with decreased IFN-γ production[6].

Heparmin® is a blend of extracts containing 100 mg of Paliasa extract (Kleinhovia hospita), 75 mg of Temu lawak (Curcuma xanthorriza) extract, 100 mg of black cumin (Nigella sativa) extract, 100 mg of snakehead fish (Ophiocephalus striatus) extract and Sambiloto (Andrographis). paniculata) 100 mg which functions as an antioxidant, hepatoprotector, anti-inflammatory, and immunomodulator[7]. The Kleinhovia Hospita plant has anti-cancer, anti-diabetic, anti-oxidant and hepatoprotective functions[8]. Curcumin, a content of Curcuma Xanthoriza, can be used as an alternative hepatoprotector in chronic hepatitis patients[9]. Nigella Sativa plants function as hepatoprotectors and play a role in oxidative stress[10]. Based on the data above, this study was conducted to determine the benefits of giving heparmine to patient tuberculosis drug resistance for 2 months which aims to minimize DILI side effects, increase the patient's tuberculosis drug resistance healing response, reduce the lost to follow-up rate and increase adherence to patient tuberculosis drug resistance treatment.

Method

Participants in this study were patients diagnosed with drug resistance tuberculosis who met the inclusion and exclusion criteria. The participant's inclusion criteria included drug resistance tuberculosis patient with STR therapy regimen, participants had normal liver function without accompanying chronic hepatitis (chronic hepatitis B and chronic hepatitis C), and aged 18-65 years. The participant exclusion criteria included patients with HIV/AIDS, patients who were pregnant or breastfeeding, patients with kidney problems and patients allergic to Heparmin®. Participants who were willing to take part in the research first received an explanation regarding the research objectives and filled out informed consent.

The design of this study used a pre-experimental design which was carried out in two locations, namely Dr. Soetomo General Academic Hospital and Mohammad Noer Hospital. The number of participants in this study were 14 participants who were obtained using consecutive sampling. Retrieval of participant data included participant characteristics, IFN-γ, and liver function (aspartate aminotransferase/AST, alanine transaminase/ALT, direct bilirubin, and total).

Heparmin® is a standard herbal medicine containing a mixture of Paliasa extract (Kleinhovia hospita) 100 mg, Temu lawak extract (Curcuma xanthorriza) 75 mg, black cumin extract (Nigella sativa) 100 mg, snakehead fish extract (Ophiocephalus striatus) 100 mg and Sambiloto (Andaphrogris) paniculata) 100 mg which functions as an antioxidant, hepatoprotector, anti-inflammatory and immunomodulator.

Interferon Gamma (IFN-γ) is a cytokine produced by T helper-1 (Th1) cells which plays an important role in eliminating Mycobacterium tuberculosis. The examination was carried out using venous blood plasma which was measured using the ELISA kit IFN-γ Bioassay Technology Laboratory brand which was expressed in units of ng/ml with a standard value of 1-400 ng/ml.

The liver function value is the value of transaminase levels in serum measured using the spectrophotometric method and the serum bilirubin value measured by the diazo reaction (erlich reagensia) which produces pink azobilirubin with the highest normal value of AST and ALT for man 0-50 u/l, woman 0-35 u/l, direct bilirubin values 0-0.2 mg/dl, and total bilirubin 0.1-1 mg/dl.

Data analysis used SPSS 23.0 to prove the effect of heparmine administration on IFN-γ and liver function values. The analysis used to test before and after giving heparmine used paired t test for normally distributed data and using the Wilcoxon signed rank test for data that were not normally distributed. The relationship between the two variables is declared significant if the p value is <0.05.

Result

Participant characteristics

Most of the participants were male as much as 71% and most were in the age range of 21-30 years (36%) and 41-50 years (36%). At most high school educated participants were 6 participants (43%). Most of the participants had weight in the normal category (57%) and the most participants showed the results of GenXpert mycobacterium tuberculosis Detected Medium as much as 43%. Most of the participants did not have comorbids (57%; Table 1).

Acid resistant bacteria and Sputum Culture

The results of the acid resistant bacteria examination were negative as many as 8 participants, after 1 month of using Heparmin® as many as 11 participants were declared negative (p = 0.375) and 2 months of using Heparmin® as many as 13 participants were stated negative (p = 0.125). The results of the Culture Sputum examination were negative as many as 3 participants, after 1 month of using Heparmin® as many as 11 participants were declared negative (p = 0.008) and 2 months of using Heparmin® as many as 14 participants were stated negative (table 2).

Comparison of IFN-γ, AST, ALT, direct and total bilirubin levels before and after administration of Heparmin®

The mean value of IFN-γ levels before heparmin administration was 90.61 ± 9.11 ng/ml with the lowest value 76 ng/ml and the highest value 107.5 ng/ml. The mean of IFN-kadar levels after heparmine administration was 91.11 ± 8.60 ng/ml with the lowest value being 78.6 ng/ml and the highest value being 108.6 ng/ml (p = 0.876). The median value of SGOT before giving Heparmin® was 28.5 (15-58) u/l and after giving Heparmin® was 33.5 (19-95) u/l (p = 0.033). The mean value of ALT before giving Heparmin® was 32.36 ± 19.17 u/l with the lowest value of 15 u/l and the highest value of 87 u/l. After giving Heparmin®, the mean ALT value was 40.21 ± 33.93 with the lowest value 12 u/l and the highest value 140 u/l (p = 0.180). The mean value of direct bilirubin before administration of Heparmin® was 0.13 ± 0.12 mg/dl with the lowest value of 0.03 mg/dl and the highest value of 0.53 mg/dl. The mean direct bilirubin after administration of Heparmin® was 0.14 ± 0.07 mg/dl with the lowest value of 0.06 mg/dl and the highest value of 0.31 mg/dl (p = 0.821). The mean total biliruin before giving Heparmin® was 0.39 ± 0.23 mg/dl with the lowest value 0.20 mg/dl and the highest value 0.94 mg/dl. The mean total biliruin after administration of Heparmin® was 0.46 ± 0.20 mg/dl with the lowest value 0.23 mg/dl and the highest value 0.81 mg/dl (p = 0.141; table 3).

Table 1. Characteristics of participant

Variable	n (%)
Gender	
Male	10 (71)
Female	4 (29)
Age	
21-30 years old	5 (36)
31-40 years old	3 (21)
41-50 years old	5 (36)
51-60 years old	1 (7)
Education	
Primary school	2 (14)
Junior high school	4 (29)
senior high school	6 (43)
College	2 (14)
BMI (Kg/m2)	
Underweight (<18.5)	6 (43)
Normal (18.5-24.9)	8 (57)
Overweight (>25)	0 (0)
GeneXpert	
Very Low	3 (21)
Low	3 (21)
Medium	6 (43)
High	2 (14)
Classification of treatment cases	
Premiere	7 (50)
Re-treatment	7 (50)
Comorbid	
None	8 (57)
Diabetes Mellitus Type 2	6 (43)

Table 2. Results of Acid-Resistant Bacteria and BTA Culture After Giving Heparmin®

Variable	Baseline	Month 1		Month 2	
		n	p	N	p
Sputum of acid resistant bacteria					
Negative	8	11	0.375	13	0.125
Positive	6	3		1	
Culture Sputum					
Negative	3	11	0.008	14	-
Positive	11	3		0	

Table 3. Comparison of IFN-γ Levels and liver function before and after Heparmin® Administration

Biomarker	Heparmin®		p
	Pretest	posttest	
IFN-γ	90.61 ± 9.11	91.11 ± 8.60	0.876
SGOT	28.5 (15.00-58.00)	33.5 (19.00-95.00)	0.033*
SGPT	32.36 ± 19.17	40.21 ± 33.93	0.180
Bilirubin direct	0.134 ± 0.12	0.141 ± 0.07	0.821
Bilirubin total	0.39 ± 0.23	0.46 ± 0.20	0.141

Discussion

TB Multiple Drug Resistance patients who received STR treatment and given Heparmin® can increase the conversion of acid-resistant bacteria and sputum culture in the first and second months. The number of acid-resistant bacteria decreased rapidly after starting anti-tuberculosis drug treatment, 80-85% of tuberculosis patients lung becomes noninfectious after about 2 weeks of treatment. The supplementation of black cumin (Nigella Sativa) together with category I anti-tuberculosis drugs can significantly increase the sputum conversion of positive acid-resistant bacterial pulmonary TB patients at the end of the second week of the intensive phase(11). Several indicators were used to evaluate the progress or success of multiple drug resistant tuberculosis control, including the time of the conversion month, namely the change in the culture of acid-resistant bacteria and sputum culture from positive to negative(1).

There is a relationship between sputum conversion examination with the predicted cure, namely the occurrence of sputum conversion in the first month of treatment is associated with 89% of treatment success, where the success of therapy in the early stages is needed to ensure the success of the next stage. The national program establishes monthly follow-up examinations during treatment(12). The results of a patient's smear sputum smear are influenced by various factors, such as the patient's ability to have an adequate cough, a small volume of sputum (ideal amount for testing 5-10 ml), consistency of sputum (mucoid or purulent). The conversion of BTA is highly influenced by the right drug mix, the right dose, the regularity of taking medication, the nutritional status and the patient's immune status(11).

Mycobacterium tuberculosis is a facultative intracellular bacteria. In dealing with intracellular microorganisms, the immune response that occurs is the cellular immune response. Helper-1 (Th1) T cells play a very important role in the body's defense system, especially in dealing with intracellular bacterial infections. One of the cytokines produced by Th1 cells is IFN-γ which plays an important role in eliminating mycobacterium tuberculosis. In TB patients there was a decrease from Th1 which was marked by low IFN-γ(11). Patients with MDR TB showed low activity of T cytotoxic cells (CTL). A study conducted by Fortes et al found that IFN-γ levels in MDR TB patients (553 ± 11 pg/ml) were lower than in non-MDR TB patients (1179 ± 163 pg/ml)(13).

The content of Ophicephalus Straiatus which contains albumin and Nigella Sativa which contains Thymoquinone in heparmin has activity as an immunomodulator which functions to repair or rebuild an imperfect or dysfunctioning immune system(14). Albumin can bind IFN-γ to increase the potentiation of IFN-γ so that it can be more competitive against

the inhibition of Suppressor of Cytokine Signaling 1 (SOCS1) induced by mycobacterium tuberculosis(15). Nigella Sativa can increase the T helper cell population in stimulating cytokines that play a role in the cellular immune system(11).

In this study, the mean value of IFN-γ levels before administration of Heparmin® was 90.61 ± 9.11 ng/ml while the IFN-γ levels after administration of Heparmin® were 91.11 ± 8.60 ng/ml (p = 0.876). In contrast to previous studies which stated that Nigella Sativa together with OAT Category I can significantly increase IFN-γ levels(11). There are several reasons that may cause no significant difference between IFN-γ levels before and after heparmine administration, among others, because the study subjects had varying BMI values. This variation in the BMI value can make a difference in the effectiveness of IFN-γ reduction to the administration of heparmine which contains Ophicephalus Straiatus and Nigella Sativa. Malnutrition and low BMI can reduce the immune system and lead to recurrence of infectious diseases(15).

Another possibility is that there are external factors that cannot be controlled by the researcher that can inhibit the IFN-γ signaling process (activation of macrophages by IFN-γ) such as the mental condition of the research subjects. In addition, there is an increase in certain cytokines that can inhibit the IFN-γ signaling process. In Micobacterium tuberculosis infection, there is an increase in IL-6 levels in the blood which can inhibit the macrophage response to IFN-γ. IL-6 can increase SOCS1 which is the main obstacle in the IFN-γ signaling process(15).

Hepatotoxicity is an increase in the levels of AST/ALT and bilirubin in the blood. AST/ALT is an enzyme related to liver function and glucose conversion that is usually found in the mitochondria of liver cells. Different levels of this enzyme can indicate different conditions and causes. The liver uses these enzymes for amino acid metabolism and to make proteins. When liver cells are damaged, AST and ALT leak into the bloodstream and cause their levels to increase in the blood(16). Previous studies stated that there was a significant difference between AST levels between subjects who had comorbid DM and those without comorbids, while for ALT there was no significant difference between subjects with co-morbid diabetes mellitus and those without comorbids. This is in accordance with the sample of this study where 6 patients out of 14 patients had comorbid diabetes mellitus(17).

There was no significant difference between ALT values before and after Heparmin® administration in multiple drug resistant tuberculosis patients receiving STR therapy. In contrast to the research conducted by Indriya, where there was no significant difference in liver function values in the group given curcumin or the control group. Although the mean AST, ALT and total bilirubin levels in the curcumin group were relatively lower when compared to the control group, they were not statistically significant. This is because since the initial administration of anti-drug tuberculosis of multiple drug resistant, all study samples had liver function that was within normal limits. There was no incidence of DILI in this study so that the effectiveness of heparmine in reducing liver function values cannot be clearly seen. The absence of a control group also makes it unable to compare the effectiveness of heparmine in reducing liver function values(18).

AST is found in all tissues with metabolic activity and red blood cells. AST levels will increase in the blood if there is damage to liver cells, however AST is not specific only in the liver. Increased AST levels are found in myocardial necrosis, cirrhosis, liver cancer, chronic hepatitis and liver congestion. Meanwhile, ALT is the main enzyme found in liver cells and is effective in diagnosing hepatocellular destruction and a little in kidney, heart and skeletal muscle. This increase in ALT indicates medical problems such as hepatitis and necrosis (highest increase), cirrhosis of the liver, liver cancer, congestive heart failure, acute alcohol intoxication (mild increase) and the influence of drugs due to antibiotics, narcotics, hypertension, salicylates, oral contraceptives and heparin(19).

When TB patients consume anti-drug tuberculosis, it will affect their bilirubin levels. This causes indirect bilirubin to settle in the blood due to the large amount of indirect bilirubin that does not bind to albumin for the conjugation process. As for direct bilirubin, liver cell damage causes bilirubin to not be excreted out of the bile into the intestine so that it will re-enter and be absorbed into the bloodstream. Therefore, from the results of checking bilirubin levels, there will be an increase in direct and indirect bilirubin(20).

Conclusion

This study involved 14 multiple drug resistant tuberculosis patients consisting of 10 male patients (71%) and 4 female patients (29%) with the most average age 36.5 years, high school education, normal BMI, GeneXpert micobacterium tuberculosis Detected Medium, without comorbids. with primary cases and re-treatment cases having the same ratio. There was sputum culture conversion in all subjects in the second month. The mean value of IFN-γ levels before Heparmin® administration was 90.61 ± 9.11 ng/ml and after heparmine administration was 91.107 ± 8.560 ng/ml. The mean values of AST, ALT, direct and total bilirubin before and after Heparmin® administration for 2 months were still within normal limits. There was no significant difference in IFN-γ levels before and after 2 months of heparmine administration in multiple drug resistant tuberculosis patients receiving STR regimen therapy. There was no significant difference before and after heparmin administration on the value of ALT, direct bilirubin and total bilirubin in multiple drug resistant tuberculosis patients receiving STR regimen therapy. As for AST, statistically there is a difference but it is still within normal limits.

Funding: None.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1814/KEPK/II/2020).

References

1. Soedarsono S. Tuberculosis: Development of New Drugs and Treatment Regimens. Jurnal Respirasi. 2021;7(1):36-45.
2. Ahmad N, Javaid A, Syed Sulaiman SA, Afridi AK, Zainab, Khan AH. Occurrence, Management, and Risk Factors for Adverse Drug Reactions in Multidrug Resistant Tuberculosis Patients. American journal of therapeutics. 2018;25(5):e533-e40.
3. Davern TJ. Drug-induced liver disease. Clinics in liver disease. 2012;16(2):231-45.
4. Suk KT, Kim DJ. Drug-induced liver injury: present and future. Clinical and molecular hepatology. 2012;18(3):249-57.
5. Ramappa V, Aithal GP. Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management. Journal of clinical and experimental hepatology. 2013;3(1):37-49.
6. Widjaja JT, Jasaputra DK, Roostati RL. Analisis kadar interferon gamma pada penderita tuberkulosis paru dan orang sehat. Jurnal Respirologi Indonesia. 2010;30(2):119-24.
7. Sirait RRU, Windarti I, Fiana DN. Effect of Oral Route Rhizome Temulawak (Curcuma Xanthorriza Roxb.) on Liver Damage of White Male Rats (Rattus Norvegicus) Sprague Dawley Strain Induced by Aspirin. Medical Journal of Lampung University. 2014;3(4):129-37.
8. Paramita S. Tahongai (Kleinhovia hospital L): A review of Herbal Medicine from East Kalimantan. Jurnal Tumbuhan Obat Indonesia. 2016;9(1):29-36.
9. Marinda FD. Hepatoprotective effect of curcumin in chronic hepatitis. J Majority. 2014;3(7).
10. Adam GO, Rahman MM, Lee SJ, Kim GB, Kang HS, Kim JS, et al. Hepatoprotective effects of Nigella sativa seed extract against acetaminophen-induced oxidative stress. Asian Pacific journal of tropical medicine. 2016;9(3):221-7.
11. Nurdin A, Hasan H. Pengaruh Jintan Hitam (Nigella Sativa) pada Konversi Sputum dan IFN γ Penderita Tuberkulosis Paru yang Mendapat OAT Kategori I pada Akhir Minggu Kedua Fase Intensif. Jurnal Respirasi. 2015;1(3):73-80.

12. Ahmad Z, Syafriani D, Merianson. MDR TB (multi drug resistant tuberculosis) reversi. Indonesian Journal of Chest Critical and Emergency. 2016;3(3).
13. Fortes A, Pereira K, Antas PR, Franken CL, Dalcolmo M, Ribeiro-Carvalho MM, et al. Detection of in vitro interferon-gamma and serum tumour necrosis factor-alpha in multidrug-resistant tuberculosis patients. Clinical and experimental immunology. 2005;141(3):541-8.
14. Gholamnezhad Z, Boskabady MH, Hosseini M. Effect of Nigella sativa on immune response in treadmill exercised rat. BMC Complement Altern Med. 2014;14:437-.
15. Pratama HA, Efendi E, Riyanti R. Pengaruh Ekstrak Albumin Ikan Gabus (Chana striata) terhadap Kadar IFN-γ Pasien Tuberkulosis Paru dengan Pengobatan Fase Intensif (The Effect of Albumin Snakehead Fish (Chana striata) Extract on IFN-Y of Pulmonary Tuberculosis Patients during Intens. Pustaka Kesehatan. 2016;4(2):222-8.
16. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. American journal of respiratory and critical care medicine. 2003;167(11):1472-7.
17. Reza A, Rachmawati B. Perbedaan kadar sgot dan sgpt antara subyek dengan dan tanpa diabetes mellitus. Diponegoro Medical Journal. 2017;6(2):158-66.
18. Indriya A. Pengaruh Pemberian Curcumin Terhadap Perubahan Klinis Efek Samping Pada Pasien Tuberkulosis Dalam Pengobatan Kategori 1 Fase Intensif 2019.
19. Kalma. Studi Hasil Pemeriksaan Serum Glutamic Oxalacetic Transminase dan Serum Glutamic Phyruvic Transminase pada Penderita Tuberkulosis Paru Sebelum dan Setelah Satu Bulan Mengkonsumsi Obat Anti Tuberkulosis. Media Analis Kesehatan. 2016;VII(2):7-18.
20. Yunita C, Dewi NU. Studi Analisis Kadar Bilirubin terhadap Lama Waktu Konsumsi Obat Anti Tuberkulosis (OAT) pada Penderita Tuberkuosis Paru. Jurnal Media Analis Kesehatan. 2019;10(1).