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PREVALENCE OF LIVER FIBROSIS DETECTED BY TRANSIENT ELASTOGRAPHY IN RHEUMATOID ARTHRITIS PATIENTS TREATED WITH METHOTREXATE

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ABSTRACT

Background: Methotrexate is the main drug used to treat rheumatoid arthritis (RA), but it can cause liver fibrosis. In the past, liver biopsy was required to assess the degree of fibrosis, but nowadays a non-invasive test known as transient elastography is available and has been shown to be equally effective. We aimed to determine the prevalence and risk factors of liver fibrosis in rheumatoid arthritis patients treated with methotrexate using TE. **Methods:** This study was conducted to evaluate liver stiffness using TE in RA patients without pre-existing liver diseases who had taken a cumulative dose of methotrexate of more than 1,500 mg over a period of more than 3 years. Patients with TE of more than 7.1 kPa and more than 9.5 kPa were classified as having significant and advanced fibrosis respectively. **Results:** A Total of 100 patients were included. The average duration of methotrexate exposure was 8.2±4.2 years and the average cumulative dose was 4,227.5±1,925.1 mg. Both significant fibrosis (11%) and advanced fibrosis (5%) were found in our study. Only BMI and CAP were found to be risk factors. **Conclusions:** Future prospective research may need to exclude NAFLD in order to assess the effects of MTX alone.

KEYWORDS: *Methotrexate, Liver fibrosis, Transient elastography, Fibroscan.*

INTRODUCTION

Rheumatoid arthritis (RA) is a common disease in the general population. The main first-line treatment for RA nowadays involves disease modifying anti-rheumatic drugs (DMARDs) which should be used as early as possible after RA is diagnosed. Methotrexate (MTX), one of these DMARDs, is the first-line treatment for RA because it is highly effective in controlling disease activity; however. despite its many therapeutic benefits, it has been reported to have some serious side effects such as bone marrow suppression, pneumonitis, oral ulcers, gastrointestinal tract problems and also hepatotoxicity.[1,2]

Many methotrexate-related adverse hepatic side effects such as transient liver enzyme elevation, hepatic fibrosis and even cirrhosis, have been reported in initial studies, and the recommended dosage has therefore been reduced to once weekly in order to alleviate these problems, resulting in a dramatic decrease in methotrexate-related hepatotoxicity.[3] Recent data have shown that RA patients who take methotrexate have a lower incidence of forms of severe hepatotoxicity, such as liver fibrosis and cirrhosis, than psoriatic patients who take the same drug; however, most rheumatologists still have concerns about its use even though severe side effects are found in only 1 in 1000 cases of patients with rheumatoid arthritis exposed to methotrexate for longer than 5 years.[3,4]

In the past (in the pre-elastography era), assessment of degree of liver fibrosis in patients taking methotrexate could only be done by liver biopsy[5] which, as mentioned earlier, is a high-risk procedure that can result in serious complications such as abdominal pain, bleeding, injury to other adjacent organs or even death.[6] Nowadays, non-invasive tests are available, including blood tests for liver fibrosis or measurement of liver fibrosis using transient elastography (TE).[4,7-9] which has been shown in many studies to be as effective as liver biopsy.[4] Studies which have aimed to assess the degree of hepatic fibrosis from methotrexate using TE have mostly been performed in psoriasis patients rather than RA ones. In Thailand, there has only been one study of TE in evaluating liver tissue in psoriatic patients[10], and no research has been performed with RA groups. The data that is available usually concerns groups of psoriatic patients, who tend to experience more hepatic side effects after using this medication.[4] Therefore, our study was designed in order to determine the prevalence of hepatic fibrosis and its precipitation in rheumatoid arthritis patients taking long-standing methotrexate.

MATERIALS AND METHODS

This was a cross-sectional study which collected data prospectively from January to December 2018. The study population consisted of RA patients from the outpatient section of the rheumatology clinic in Rajavithi Hospital. The study population who fulfilled the inclusion criteria were included after receiving clear informed consent. All had been exposed to MTX with a cumulative dosage of more than 1.5 grams over a period of more than 3 years. Physical examination, liver biochemical test and viral markers for hepatic B and C were done to exclude co-existing liver conditions. Patients who consumed more than 20 grams of alcohol per day or equivalent in the preceding 10 years were excluded. Liver fibrosis was assessed by well-trained nurses using transient elastography (Fibroscan® 502 touch, Echosen, Paris). Ten validated measurements, a success rate of at least 60%, and an interquartile range (IQR)/median of less than 30% were considered acceptable. We defined TE >7.1 as significant fibrosis and TE > 9.5 as advanced fibrosis. The primary objective of our study was to find the prevalence of significant and advanced liver fibrosis associated with long term MTX therapy in patients with RA, while the secondary aim was to determine risk factors associated with significant liver fibrosis. Our study was approved by the ethics committee of Rajavithi Hospital.

Inclusion criteria

Patients who:

1. had rheumatoid arthritis and were treated with methotrexate at Rajavithi Hospital
2. were aged 18 – 70 years
3. had received methotrexate treatment for > 3 years
4. had received a cumulative dose of methotrexate > 1.5 grams

Exclusion criteria

Patients who:

1. had cirrhosis or other known pre-existing liver disease
2. had chronic viral hepatitis B or C
3. were males with a history of consuming more than 20 g of alcohol per day (2 standard drinks per day) or females who drank more than 10 g per day (1 standard drink per day)
4. were pregnant

The sample size of this study was calculated using finite population proportion.[11] Using type I error = 0.0068 and power of 80%, the required sample size was calculated at 97, and 10% was added to compensate for missing data; thus we included 107 patients in this study.

Analysis was performed with the SPSS version 23.0 (SPSS, Chicago, IL, USA). Data were presented as mean ± standard deviation (SD) or median (range) or median (IQR) for continuous variables and number (%) for categorical variables. Differences in the frequencies of events between groups were analyzed using Chi-square test or Fisher's exact test. Independent t-test or Mann-Whitney test were used to compare continuous variables between groups. Pearson correlation analysis was used to test for correlation of normal variables, whereas Spearman correlation analysis was used for variables not showing a normal distribution. A p-value of less than 0.05 was considered statistically significant.

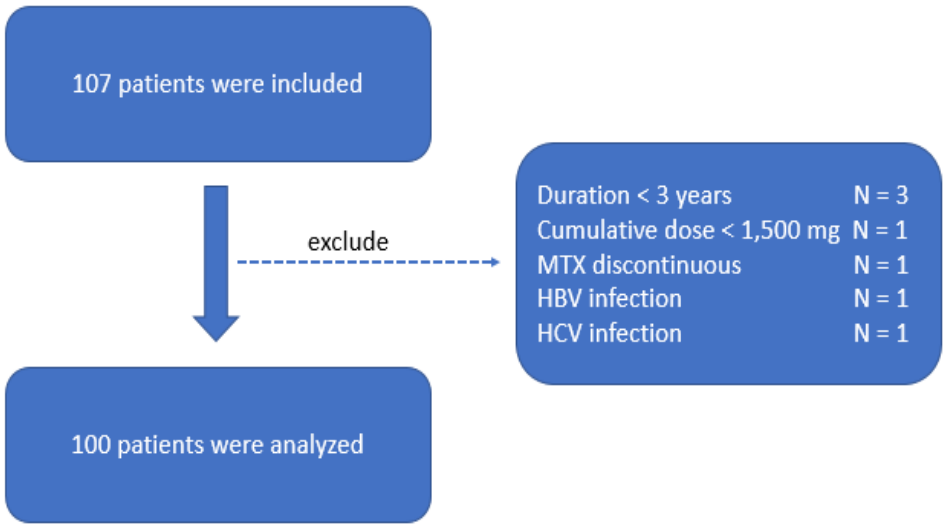


Figure 1 : Flow chart of study

RESULTS

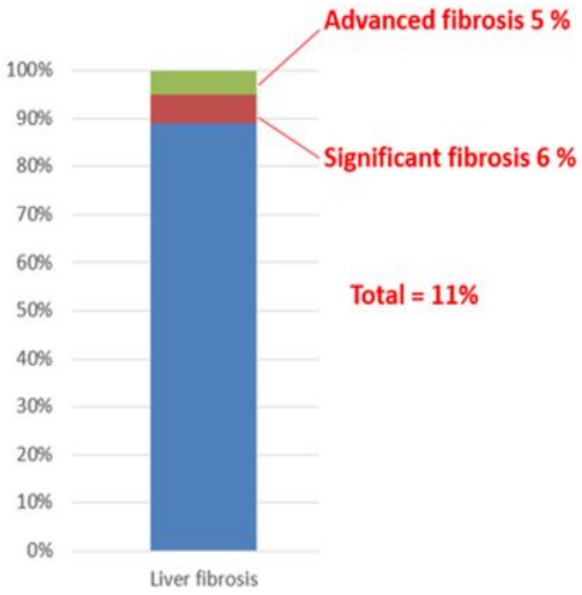
Demographic data

Table 1 and Figure 1 show a total 107 patients; however, 7 of these were excluded, leaving 100 patients for data analysis, which exceeded the required number derived from sample size calculation. The average age of the population was 54.1 ± 10.9 years. The youngest patient in this study was 23 years old, the oldest was 70 years old, and females predominated (89%). The average weight, height, body mass index (BMI) and underlying diseases are shown in Table 1.

Table 1: Demographic data.

	Total (n = 100)	
	n	%
Age (y)		
< 60	66	66.0%
≥ 60	34	34.0%
Mean±S.D.	54.07±10.89	
Sex		
Male	11	11.0%
Female	89	89.0%
BMI (kg/m²)		
<18.5	4	4.0%
18.5-24.9	59	59.0%
25-29.9	27	27.0%
≥ 30	10	10.0%
Mean±S.D.	24.28±4.23	
Underlying disease		
Hypertension	38	38.0%
Dyslipidemia	30	30.0%
Diabetes	13	13.0%
Cumulative dose (mg)		
Mean±S.D.	4227.47±1925.10	
Duration (years)		
Mean±S.D.	8.18±4.26	
CAP		
Mean±S.D.	223.39±52.50	

Figure 2: Prevalence of liver fibrosis.



The cumulative doses of methotrexate in our study were as follows: the mean dose was 4,227.5 mg ± 1929.10, the minimum was 1,505 mg and the maximum was 10,625 mg the mean duration of methotrexate exposure average was 8.2 ± 4.2 years, and range was 3 to 24 years (Table 1). The majority of our patients had also taken other medications (DMARDs and other drugs), but none of these showed any statistically significant difference when comparing the groups with and without significant fibrosis (Supplementary Table 1).

Table 2: Comparison of risk factors between TE ≥ 7.1 and < 7.1 kPa.

	Total (n = 100)		Transient elastography				p-value
			≥ 7.1 (n = 11)		< 7.1 (n = 89)		
	n	%	n	%	n	%	
Age (y)							
< 60	66	66.0%	11	100%	55	61.8%	
≥ 60	34	34.0%	0	0%	34	38.2%	
Mean±S.D.	54.07±10.89		53.27±5.73		54.17±11.38		0.384[M]
Sex							0.100
Male	11	11.0%	3	27.3%	8	9.0%	
Female	89	89.0%	8	72.7%	81	91.0%	
BMI (kg/m²)							0.032*
<18.5	4	4.0%	1	9.1%	3	3.4%	
18.5-24.9	59	59.0%	3	27.3%	56	62.9%	
25-29.9	27	27.0%	4	36.4%	23	25.8%	
≥ 30	10	10.0%	3	27.3%	7	7.9%	
Mean±S.D.	24.28±4.23		26.30±5.41		24.03±4.02		0.114[M]
Underlying disease							
Hypertension	38	38.0%	5	45.5%	33	37.1%	0.744
Dyslipidemia	30	30.0%	3	27.3%	27	30.3%	1.000
Diabetes	13	13.0%	2	18.2%	11	12.4%	0.633
Cumulative dose (mg)							
Mean±S.D.	4227.47±1925.10		3639.09±1975.96		4300.19±1917.54		0.243[M]
Duration (years)							
Mean±S.D.	8.18±4.26		7.00±4.40		8.33±4.24		0.184[M]
CAP							
Mean±S.D.	223.39±52.50		263.09±81.36		218.48±46.12		0.100[M]

p-value < 0.05 was considered statistically significant.

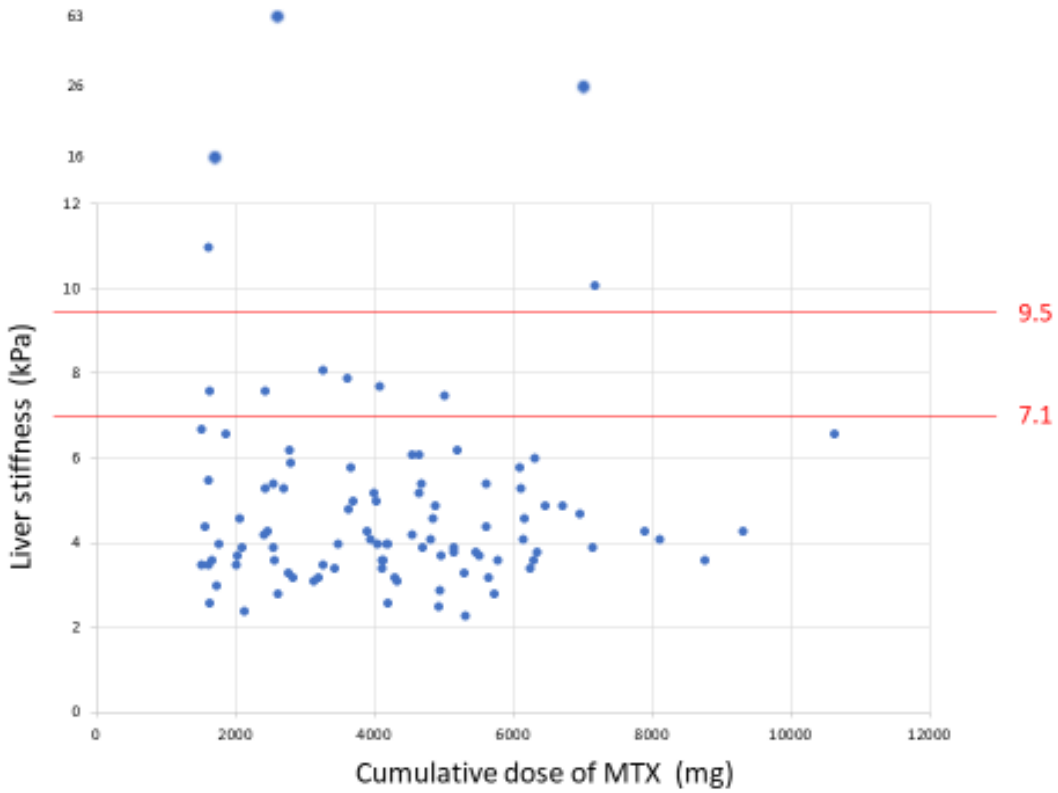
Table 3: Comparison of risk factors between TE ≥ 9.5 and < 9.5 kPa.

	Total (n = 100)		Transient elastography				p-value
			≥ 9.5 (n = 5)		< 9.5 (n = 95)		
	n	%	n	%	n	%	
Age (y)							
< 60	66	66.0%	5	100%	61	64.2%	
≥ 60	34	34.0%	0	0%	34	35.8%	
Mean±S.D.	54.07±10.89		53.40±6.11		54.11±11.10		0.601[M]
Sex							0.449
Male	11	11.0%	1	20.0%	10	10.5%	
Female	89	89.0%	4	80.0%	85	89.5%	
BMI (kg/m²)							0.067
<18.5	4	4.0%	0	0%	4	4.2%	
18.5-24.9	59	59.0%	1	20.0%	58	61.1%	
25-29.9	27	27.0%	2	40.0%	25	26.3%	
≥ 30	10	10.0%	2	40.0%	8	8.4%	
Mean±S.D.	24.28±4.23		28.41±4.67		24.06±4.11		0.045[M] *
Underlying disease							
Hypertension	38	38.0%	3	60.0%	35	36.8%	0.365
Dyslipidemia	30	30.0%	3	60.0%	27	28.4%	0.158
Diabetes	13	13.0%	2	40.0%	11	11.6%	0.125
Cumulative dose (mg)							
Mean±S.D.	4227.47±1925.10		4009±2765.50		4238.97±1890.78		0.728[M]
Duration (years)							
Mean±S.D.	8.18±4.26		6.60±3.65		8.26±4.29		0.324[M]
CAP							
Mean±S.D.	223.39±52.50		314.80±60.68		218.58±47.73		0.004* [M]

p-value < 0.05 was considered statistically significant.

Results of liver fibrosis

The average liver fibrosis was 5.5 ± 6.6 kPa, while the minimum and maximum values were 2.3 and 63.9 kPa respectively. Significant fibrosis (TE ≥ 7.1 kPa) was found in 11% of subjects and advanced fibrosis (TE ≥ 9.5 kPa) was found in 5% (Figure 2).

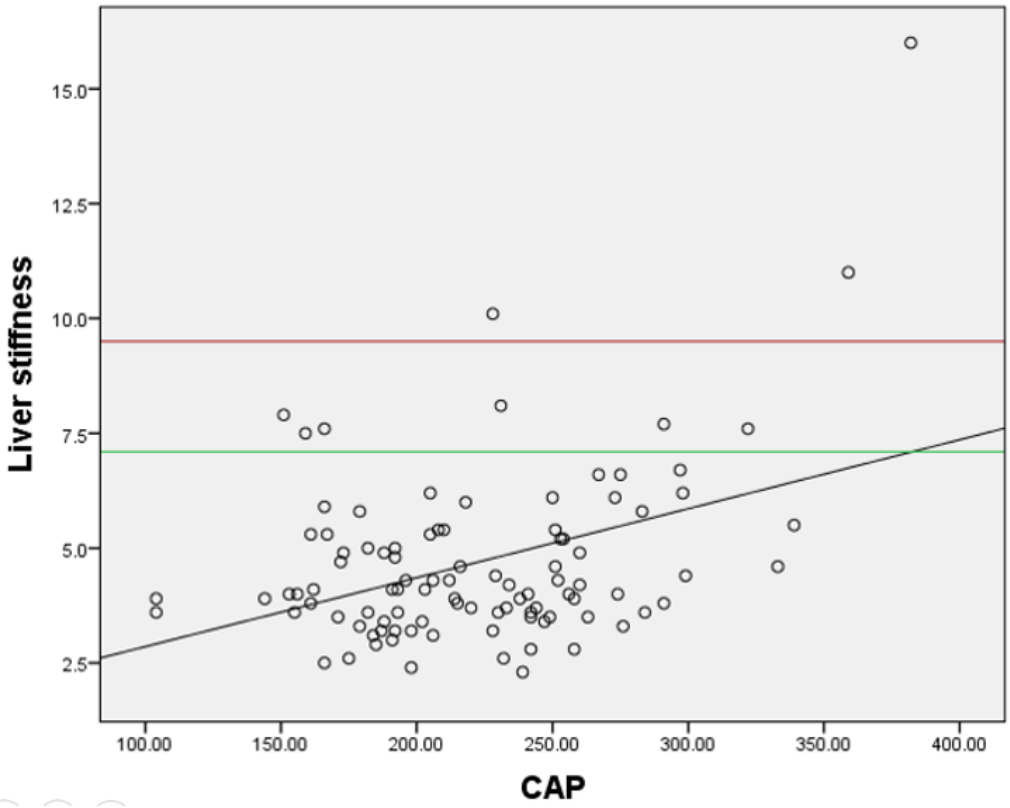


r = -0.49, P = 0.626.

Figure 3: Correlation between liver stiffness and cumulative dose.

Data displayed in Tables 2 and 3 and Figure 3 show that age, gender, underlying disease, cumulative dose and duration of methotrexate treatment were not associated with either significant or advanced fibrosis. BMI was the only risk factor that was statistically significantly different between the groups with or without significant fibrosis (TE ≥ 7.1 VS TE < 7.1 kPa) and the groups with or without advanced fibrosis (TE ≥ 9.5 VS TE < 9.5 kPa); in addition, Controlled Attenuated Parameters (CAP) was shown to be correlated with liver fibrosis (r = 0.317, p = 0.001) (Figure 4). The mean CAP showed significant differences between the group with advanced fibrosis compared to the group without it (Table 3), with the mean CAP being significantly higher in the advanced fibrosis group (table 3), while

analysis of the association between CAP and the cumulative dose of methotrexate did not show any correlation (r = -0.003, p = 0.978) (Supplementary 2).



r = 0.317, P = 0.001.

Figure 4: Correlation between liver stiffness and CAP.

DISCUSSION

A total of 100 RA patients treated with long-term methotrexate were included in our study. The prevalence of significant fibrosis (TE ≥ 7.1 kPa) was 11% and that of advanced fibrosis (TE ≥ 9.5 kPa) was 5%. Research performed by Chong Meng Yeo, et al[12] focused on psoriasis patients treated with methotrexate and used liver biopsy for liver fibrosis assessment. They reported a prevalence of liver fibrosis of 12%, which is similar to the findings of our study, adding supporting evidence to confirm the efficacy and reliability of TE in liver fibrosis assessment; furthermore, as TE is much safer than liver biopsy, it is preferable as an evaluation tool.

Our study did not detect any correlation between liver fibrosis and various risk factors such as age, gender, comorbidities, cumulative dose or duration of methotrexate use; in particular, cumulative dose and duration of methotrexate, which have long been thought to be strong

risk factors of liver fibrogenesis, were not found to be correlated in our study. High BMI, on the other hand, was correlated to both significant hepatic fibrosis (TE ≥ 7.1 kPa) and advanced fibrosis (TE ≥ 9.5 kPa). Furthermore, the controlled attenuated parameter (CAP) from the TE, which represents the degree of hepatic steatosis, was found to be correlated with hepatic fibrosis, while no correlation was found between CAP and accumulative dose of methotrexate. Hepatic fibrosis found in our study was caused by metabolic syndrome and hepatic steatosis, similar to nonalcoholic steatohepatitis (NASH). Methotrexate did not induce hepatic fibrogenesis.

Compared to most previous research, the prevalence rates of liver fibrosis in patients exposed to longstanding methotrexate in this study were quite varied, ranging from 3.4- 37.7%. The difference possibly stems from different TE cut-points and the heterogenicity of study populations. The prevalence of liver fibrosis in our study was very similar to that found in one carried out in Thailand by Pongpit J, et al[10] (10.9%), which included psoriasis patients with methotrexate treatment. Meanwhile, a study of RA patients from Korea by Park SH, et al[13] showed a much lower prevalence of liver fibrosis (Table 4); therefore, there is a possibility that race and other unknown factors (i.e. environment, drugs, food etc.) may affect liver fibrosis.

Table 4: Comparison of results of our study and others.[9,10,13-15]

Study	Disease	% Fibrosis	Cut point TE	Cumulative dose	Factors
Our study	RA	11%	7.1	X	BMI
Park SH , 2010	RA	3.4%	7.8	X	AST to ALT ratio , APRI , Haptoglobin
Kumar A , 2018	RA	7.5%	7.2	X	-
Laharie D , 2017	RA, PsA, CD	6.0%	7.9	X	BMI , Alcohol used
Pongpit J , 2016	PsA	10.9%	7.0	X	Waist circumference , AST level ,DM
Talme T , 2017	PsA	37.7% 9.0%	6.5 11.5	X	BMI , DM

Our findings revealed that CAP was associated with liver fibrosis, implying that patients with fatty liver have a higher chance of developing liver fibrosis; therefore, it is possible that the important factor leading to liver fibrosis is not cumulative dose of methotrexate as previously believed, but metabolic syndrome. Apart from our study, previous research has also demonstrated a correlation between liver fibrogenesis and features of metabolic syndrome such as diabetes, hyperlipidemia and obesity.[9,10,12,14] This research confirms the findings of past studies of liver fibrosis in patients taking MTX which found that it was caused by metabolic factors. In fact, MTX-induced steatohepatitis may not even exist at all, and all patients are possibly affected by NAFLD; however, clearer conclusions need to be drawn from future studies.

Our research is the first in Thailand to investigate the prevalence and risk factors of liver fibrosis in RA patients exposed to MTX using TE. The study included patients who had been treated for a long time and had very high mean cumulative doses, ensuring that their methotrexate exposure was more than sufficient. There were some limitations of our study: it had too few subjects with advanced fibrosis, so that analysis of risk factors may be inadequate; and it did not exclude patients with NAFLD, which is a strong confounder in hepatic fibrogenesis. Larger sample sizes with NAFLD exclusion are therefore required for more accurate results.

In conclusion, BMI and CAP were risk factors associated with liver fibrosis, while cumulative dose of methotrexate was not.

Conflict of interest

The authors have no conflict of interest to disclose.

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