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Study Protocol and Statistical Analysis Plan

Using hydroxychloroquine to treat nonalcoholic steatohepatitis

Study Title：Using hydroxychloroquine to treat nonalcoholic steatohepatitis

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Study Director：Chung- Jui Huang

Objective：To examine whether hydroxychloroquine can treat nonalcoholic steatohepatitis effectively.

Abstract：

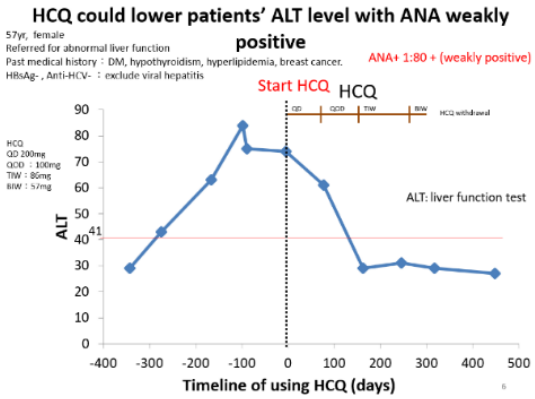
Nonalcoholic steatohepatitis (NASH) is a serious type of nonalcoholic fatty liver disease(NAFLD), which is characterized by lobular inflammation and apoptosis resulting from hepatic steatosis in the absence of excessive alcohol consumption[1]. If NASH are not controlled well, it will advance to liver fibrosis, cirrhosis[1], and even hepatocellular carcinoma[2]. However, there is no approved treatments currently.

According to guidance of American Association for the Study of Liver Diseases (AASLD) and a few studies, vitamin E and pioglitazone are two potential pharmacologic therapies for NASH. They have some therapeutic effects; however, they also have some safety concerns[3]. Therefore, those two drugs have not been wildly used in clinical practices. In addition to vitamin E and pioglitazone, many drugs are being tested in clinical trials. However, promising results have not been revealed yet.

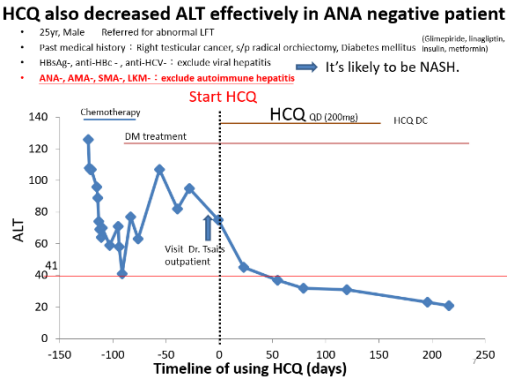
Recent clinical and experimental data suggested that hydroxychloroquine (HCQ) might improve metabolic profiles in patients or animals with obesity-related metabolic disorders[4, 5]. HCQ might also relieve liver inflammation in experimental animals with steatohepatitis[5]. As an inhibitor of endocytosis and autophagy with therapeutic effects in autoimmune disorders[6], it is reasonable to speculate that HCQ might also relieve liver inflammation in patients with steatohepatitis. Therefore, HCQ has been anecdotally prescribed to patients with steatohepatitis in our clinic. Excitingly, almost all those with HCQ treatment had immediate reduction of their liver enzyme levels, indicating instant relief of their steatohepatitis. Effects of HCQ were similar in those with negative anti-nuclear antibody (ANA) compared to those with weakly positive ANA (Figure A, B), suggesting that those are specific steatohepatitis-targeting rather than autoimmune-targeting effects from HCQ.

Thus, we aim to clarify whether HCQ relieves nonalchoholic stetohepatitis by reviewing medical records from our out-patient-clinic patients who accept the treatment of hydroxychloroquine (Plaquenil®).

A



B



Design：Retrospective study

Review medical records from patients who accept the treatment of hydroxychloroquine (Plaquenil®), find those with nonalcoholic steatohepatitis and observe the effects of hydroxychloroquine on them.

Methods：

Step 1：screen out our targeted group：patients with nonalcoholic steatohepatitis

1. We have made a request to Information Technology Office of National Taiwan University Hospital, and this request is about the list of our out-patient-clinic patients who accept the treatment of hydroxychloroquine.
2. Collect and review the medical records, ultrasound and blood tests of patients in the abovementioned list and screen out those with nonalcoholic steatohepatitis based on the following criteria.

Inclusion criteria：

Our patients who taking hydroxychloroquine and match the following criteria

A. Patients with fatty liver, based on the abdominal ultrasound report of which the date is the closest to the initial date of using HCQ.
B. ALT level of 2.5 and 0.5 month prior to hydroxychloroquine treatment > 41 U/L
C. With ALT result which is tested in 3 months after using HCQ.
D. The initial date of taking HCQ is prior to September, 26, 2022, which is the date we got the list from Information Technology Office of National Taiwan University Hospital.

Exclusion criteria：

Our exclusion criteria can be separated into loose exclusion criteria and strict exclusion criteria.

Loose exclusion criteria：patients who match the following criteria will be excluded from the above group.

A. Diseases of the biliary tract：the abdominal ultrasound report indicated the patient with stones in the biliary tract, or the patient with jaundice or his total bilirubin > 2.0 mg/dl
B. Viral hepatitis： HBV Viral Load + or HCV Viral Load +
C. Alcoholic steatohepatitis： Histroy of drinking alcohol or AST/ALT > 1.5 and GGT > 2X
D. Autoimmune hepatitis：ANA 1:80+ (above) or AMA+ anti-LKM+ or anti-SMA+
E. Wilson's disease：ceruloplasmin < 20 mg/dl

Strict exclusion criteria：patients who had ever had viral hepatitis or was being infected by virus (HBV, HCV infection：HBsAg+ or anti-HBc+ or anti-HCV Ab+) will be further excluded from the above group.

The definition of months：used in the criteria evaluation and the data analysis
Before patients used HCQ：
-0.5 month：During the periods from one month prior to the date of initial use of HCQ to that date, we define the date which is the closest to 0.5 month as -0.5 month.
-2.5 months：During the periods from four months to one month prior to the date of initial use of HCQ, we define the date which is the closest to 2.5 months as -2.5 months.
-5.5 months：During the periods from seven months to four months prior to the date of initial use of HCQ, we define the date which is the closest to 5.5 months as -5.5 months.

After patients used HCQ：
0.75 months：During the periods that patients used HCQ for 0-1.5 months, we define the date which is the closest to 0.75 months as 0.75 months.
3 months：During the periods that patients used HCQ for 1.5-4.5 months, we define the date which is the closest to 3 months as 3 months.
6 months：During the periods that patients used HCQ for 4.5-7.5 months, we define the date which is the closest to 6 months as 6 months.
9 months：During the periods that patients used HCQ for 7.5-10.5 months, we define the date which is the closest to 9 months as 9 months.
12 months：During the periods that patients used HCQ for 10.5-13.5 months, we define the date which is the closest to 9 months as 12 months.

Then, in addition to the above-mentioned criteria, we will record the following two things separately.

The first one is hemochromatosis. Hemochromatosis may also cause hepatitis, but the prevalence of this disease in Taiwan is very low. Thus, we do not list hemochromatosis in the exclusion criteria.
The second one is drug-induced hepatitis. We do not list suspected drug-induced hepatitis in the exclusion criteria since drug-induced hepatitis may not be accurately confirmed based on our current medical records. However, if we find patients using drugs which may be related to hepatitis, we will record their information separately, including the duration of using these drugs and the ALT levels after stopping using it.

Step 2：collect and analyze patients' medical information
(These patients are those with NASH and accept the treament of HCQ)
We plan to collect the following information of each patients

1. The date when each patient started to take HCQ
2. Birth date：
   To realize how old the patients were when they started to take HCQ.
3. Gender
4. Historical blood test results：
   A. ALT, AST, GGT, α-fetoprotein, platelet, FIB-4, WBC, bilirubin, albumin
      a. For recording baseline demographics
      b. To assess and analyze the status of hepatitis and liver fibrosis in each patient.

      →We plan to observe the ALT levels prior to the initial use of HCQ and ALT levels after it, and we will further analyze whether the ALT levels were decreased after patients took HCQ.
   B. HbA1c*
      a. For recording baseline demographics
      b. To assess whether patients have diabetes mellitus
   C. TG, TCHO*
      a. For recording baseline demographics
      b. To assess whether patients have hyperlipidemia

      * We need these tests to realize whether patients have these comorbidities, since these are the risk factors of NASH.

Statistical Analysis Plan

1. We plan to use the statistical function of excel and Prism to analyze our results.
2. Initially, we will make the tables about the baseline demographics of patient who in our study group (patients with NASH and accept the treatment of HCQ), which we may use excel to help us arrage and analyze the tables.

3. Then, we will check each patient's ALT levels in different month (prior to or after the initial use of HCQ), and we will use the date of initial use of HCQ as day0 and make box plots and violin plots to show the change of ALT levels with times going. We plan to use Prism to make the plots and analyze whether ALT levels were significantly decreased after pateints took HCQ.

Members of our team

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