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Serum levels of 25-hydroxyvitamin D in hepatitis C patients in Northern Norway

A retrospective cohort study

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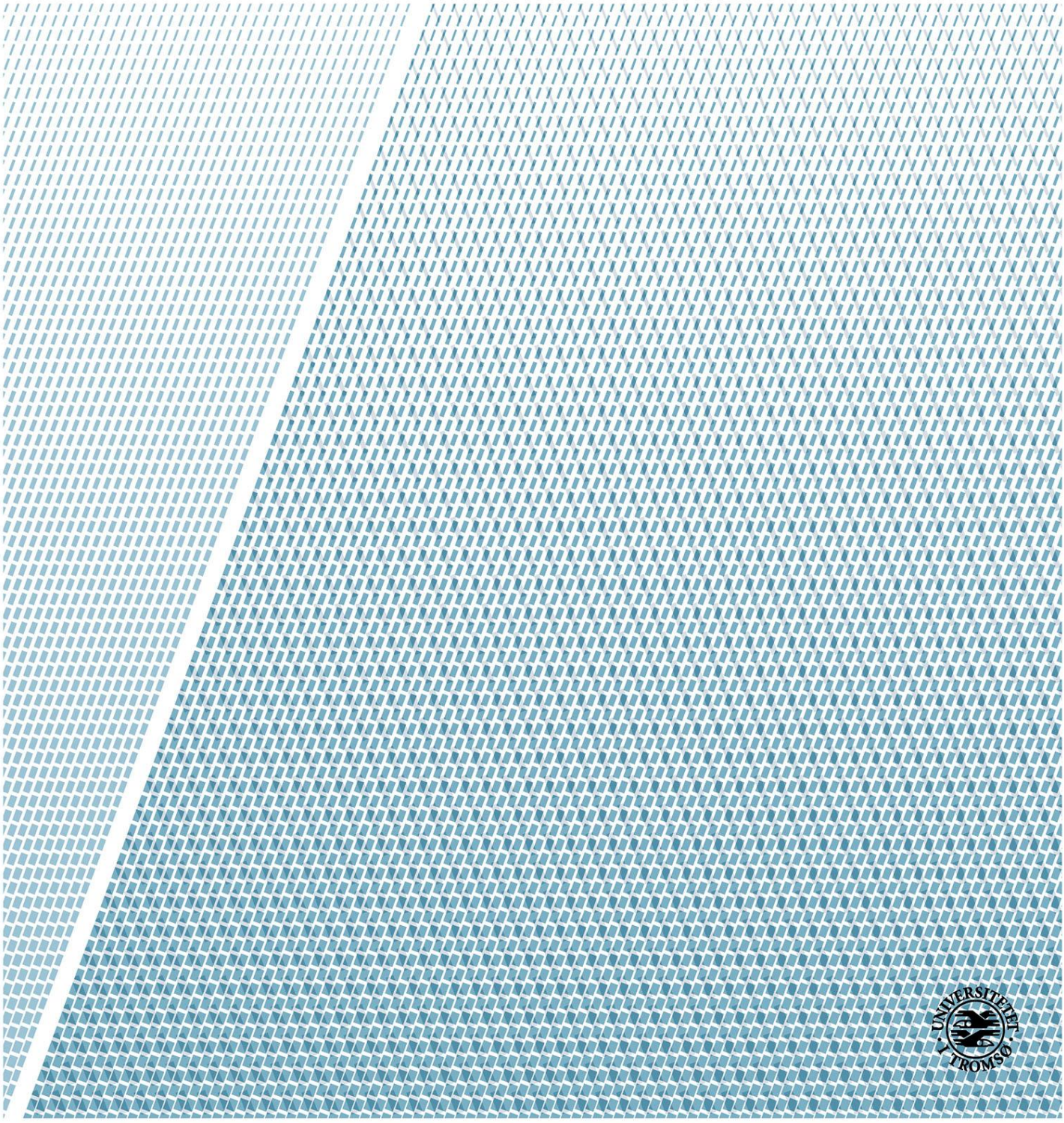

Abstract

The aim of this study was primarily to describe the vitamin D status in patients with hepatitis C virus (HCV) infection, followed up at the University Hospital of Northern Norway in Tromsø (UNN), and to study if there were any associations between vitamin D status, the level of liver enzymes, viral load, liver fibrosis, liver inflammation and sustained virological response to treatment. Additionally, we would provide a more general description of the HCV infected population in our region.

This was a retrospective cohort study, including 107 patients. The mentioned parameters were reclaimed from the computer journal system of UNN, and subsequently plotted in a spread sheet. We gave both a demographic description and did statistical analysis, using the computer program SPSS.

We found the HCV infected patients to have somewhat lower levels of vitamin D than the general Northern Norwegian population. Higher degree of inflammation tended to be related to lower levels of vitamin D, but not of statistically significance. Vitamin D and IL28B did show no significant correlation.

The level of vitamin D is of interest for HCV infected patients, possibly positively influencing the progress of disease and effect of treatment. Therefore prevention of vitamin D deficiency in this group is of importance.

Abbreviations

Hepatitis C virus, HCV; University Hospital of Northern Norway, UNN; Hepatocellular carcinoma, HCC; Single nucleotide polymorphisms, SNPs; Sustained virological response, SVR; Rapid virological response, RVR; Pegylated interferon alpha, PEG-INF; Polymerase chain reaction, PCR; Histological activity index, HAI; Norwegian regional ethical committee, REK.

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Introduction

About 170 million people worldwide, and more than 25 000 subjects in Norway, are chronically infected by the hepatitis C virus. Cirrhosis and hepatocellular carcinoma are the most frightened medical consequences of this infection, accounting for over 350 000 deaths worldwide per year. In Norway, the most frequent way of transmission is injecting drug use. Despite this, the hepatitis C population is rather heterogeneous, and subjects of all ages and different socioeconomic classes can be affected. There are very few descriptive studies of the hepatitis C population in Norway, and especially in Northern Norway, regarding for instance general health and vitamin D status.

Epidemiology

It has been estimated that about 170 million people are chronically infected by hepatitis C virus (HCV) worldwide (1). The prevalence of anti-HCV in the general adult population in Oslo was 0,7 % in 2003, with genotype 1 being found in 40 % and genotype 2 and 3 in 60 %. In the Northern Norwegian population the prevalence was estimated to be 0,24 % in 1999 (2). Because the latter was based on already known or new clinically discovered cases, rather than a general screening, it was considered a slight underestimation. 62 % and 38 % of the HCV infected persons where respectively men and women. The mean age was 33 years, with 74 % of persons being within 20-39 years. The incidence of HCV infections in Northern European countries has decreased, while the prevalence of chronically HCV infected people still increases (3). One review article describes a characteristic difference in distribution of chronically HCV infected in the Northern Hemisphere (4). It seems to follow a gradient from north to south, with an increase in prevalence towards the south. Already in Southern Italy the prevalence of HCV was 12,6 %. It also describes a shift in genotype, with genotype 1-3 in the north, and higher numbered genotypes towards the south.

HCV caused more than 8 600 deaths in Europe in 2002 (5). In the same year, on a global basis, HCV accounted for 27 % of liver cirrhosis and 25 % of hepatocellular carcinomas (HCC) (6). Out of these, cirrhosis and HCC caused respectively 211 000 and 155 000 deaths.

About 80 % of people infected by HCV, develop a chronic infection (7). The progression of fibrosis is variable, and depends amongst others on the hosts immune system, environmental

factors like alcohol abuse, coinfections with hepatitis B virus or human immunodeficiency virus and viral factors. The probability to develop liver cirrhosis after 20 years of chronic HCV infection is based on source of infection, were post-transfusion infection gives a risk of 24 %, blood-donor infection of 4 % and community-based infection of 7 %. 2,1 % and 2,7 % of chronic HCV infected patients, respectively treated with interferon alpha and not, are shown to develop HCC every 5 years (8). Apart from liver disease, HCV infection has been shown to increase the risk of chronic kidney disease. In the same Northern Norwegian population mentioned in the previous paragraph, the clinical outcomes of HCV infections were measured about 8 years later (9). Renal failure was reported in 0,2 %, decompensated liver disease in 2,9 % and HCC in 0,4 % of cases. 10,7 % of chronic HCV infected persons had a depression.

In hypoendemic areas, the mode of transmission consists mainly of behavioural factors (4). In Oslo the main source of HCV infection, was injecting drug use with 67 % (1). Post-transfusion was the main source in 6 %, while the last 27 % were unknown. In Northern Norway, injecting drug use was the main source of infection, with 67 % as well, and 3 % being caused by post-transfusion (2). Contact networks play an important part in the dynamics of transmission of HCV. One study shows that the risk of re- and superinfection increases, when being part of an injecting drug network (10). Recurrence of infection was observed in 19 % and 10 % of respectively treated and untreated HCV infected people, with previous viral suppression. Superinfection was observed in 16 % and 17 % of respectively treated and untreated HCV infected people.

The infection, without being aware of it, has been shown to cause reduction in quality of life (11). Being aware of the infection will often lead to further reduction of this, caused by public stigmatization, or perception/expectation of this. HCV infection can lead to reduced quality of life, also apart from liver disease. The side effects of treatment of HCV infection with PEG-IFN/Ribavirin can be distressing. Comorbidities, such as alcohol and drug abuse, or caused by these, could also lead to a further reduction in quality of life.

Immunology

The Hepatitis C virus was first isolated in 1989 (12). It has a positive single strand RNA genome, which encodes for one polyprotein, cleaved into three structural and seven non-structural proteins (core, E1 and E2; p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B).

Differences in core, E1 and NS5 regions, give rise to the 7 major genotypes with subtypes of HCV. The components of HCV can compromise the innate immune response at several levels, which enables it to develop and maintain chronic infections. For example the hepatic stellate cell is activated by chemokines induced by HCV infection. The hepatic stellate cell is considered to be the main contributor of liver fibrosis, by increasing the production of proteins to the extracellular matrix, which subsequently can lead to liver fibrosis and cirrhosis.

Interferon-λ3 is a cytokine that takes part in the human immune response during HCV infection (13). It is encoded at the IL28B gene. Three different single nucleotide polymorphisms (SNPs), CC, TT or CT, at rs12979860 in the ILB28 gene have become linked to different outcomes of HCV infection with or without treatment. One study found statistically significant improved outcomes of genotype 2 and 3 HCV infected persons, treated with PEG-IFN/Ribavirin, with the CC mutation, compared to CT or TT mutations (14). Lower age and infection of genotype 2 also increased the sustained virological response (SVR). Another study describes statistical significant improved rapid virological response (RVR), but not SVR, in patients infected by the genotype 3 of HCV, treated with PEG-IFN/Ribavirin, in relation to ILB28 gene mutations (15). CC mutation versus TT/CT mutations gave a RVR of 84 % vs. 61 %. A major meta analysis provided data, showing that CC statistical significantly both increases severe fibrosis and inflammation activity, compared with CT/TT (16).

Diagnosis and treatment

Even though new biomarkers of liver damage are being investigated, ALT and AST are still well established (17). ALT levels are showing low false negative and positive results, compared with liver damage. ALT is primarily located in liver tissue, while AST also is to be found in muscle, heart and brain. This makes AST less specific, but it is still considered a useful biomarker of liver damage and disease. In combination, the AST/ALT ratio index can be a cheap way to screen for liver fibrosis.

The journal of Norwegian medicine association, published in 2011 an article about recommendations for diagnosis and treatment of HCV in Norway (18). People in risk of infection should be tested for HCV antibodies. If this test is positive, an HCV-RNA polymerase chain reaction (PCR) test should be the next step, to determine ongoing infection and genotype. If positive, the degree of disease is estimated by biochemical tests, such as

ALT and AST, virological tests, such as viral load, and ultrasound of liver and spleen. If these are inconclusive, biopsy of the liver is indicated to determine liver fibrosis. As an alternative to liver biopsies, it is also possible to do non-invasive transient elastography with for example the FibroScan®. The grading of liver fibrosis with the Ishak score goes from 1 (no fibrosis) to 6 (cirrhosis, probable and definite) (19). The Histological Activity Index (HAI) describes inflammatory activity, and is graded from 0 (none) to 18 (grave). Both tables are derived from the original source, and presented in the appendix, as respectively table 3 and 4.

The traditional treatment of HCV infection consisted of Pegylated interferon-alfa (PEG-IFN) and Ribavirin (18). Patients with HCV genotype 1, with at least moderate disease, where recommended medical treatment for 48 weeks. Patients with HCV genotype 2 and 3, with or without manifested disease, where recommended medical treatment for 24 weeks. One study showed 11 % and 52 % SVR after respectively 24 or 48 weeks of treatment for HCV genotype 1, while it was 84 % and 79 % after respectively 24 or 48 weeks of treatment for HCV genotype 2/3 (20). This was considered consistent with previous studies.

Interferon analogues activated antiviral properties in the host (21). Interferon alfa-2a and -2b, bind to the interferon-alpha receptor 1 and 2, and activate the JAK-STAT signaling pathway. Whereas the mode of action of Ribavirin is unclear, it seems to give both a general stimulatory effect on T helper 1 cells, and a specific anti-HCV effect on regulatory T cells (22). Possible side effects of the combined PEG-IFN/Ribavirin treatment are among others hematological, in form of anemia, thrombocytopenia and leucopenia, skin manifestations, alopecia, arthralgias, flu-like illness and psychiatric disorders, such as depression and sleep disorders, where anemia was the main reason for cessation of Ribavirin (23). In a German trial from 2009, a total of 97,7 % of patients experienced some sort of side effect. 4,4-10,5 % experienced serious adverse effects, depending on duration of treatment, going from 12 to 24 weeks.

In addition to the traditional treatment with PEG-INF/Ribavirin, a spectrum of new drugs are being developed, inter alia direct acting antivirals (DAA's) (24). Viral enzyme inhibitors, such as the NS5A inhibitor Ledipasvir (LDV), or as the NS5B nucleotide polymerase inhibitor Sofosbuvir (SOF), are examples of this. Alone and in combination, these two drugs have shown good results. Adding Ribavirin, even improved SVR in patients with cirrhosis. In the new Norwegian guidelines for treatment of HCV, SOF/LDV have been implemented in the

treatment (25). Also drugs acting on micoRNA, and on the specific immune system, are under investigation.

Vitamin D

Vitamin D, a fat soluble steroid, is known for its importance in homeostasis of bone in the human body, mainly by regulating the level of calcium (26). 25-hydroxyvitamin D is produced by hydroxylation in the liver, and subsequently 1,25-OH-vitamin D, by hydroxylation in the kidneys. The metabolite which primarily has been used for measurement of vitamin D, is 25-hydroxyvitamin D, abbreviated 25-OH-vitamin D, and also named calcidiol. The primary sources of vitamin D are synthesis in the skin, with influence of ultraviolet radiation, and from food, such as fatty fish. Sunlight exposure is the major contributor.

The vitamin D-receptor (VDR) seems to be present in several leucocytes (27). Binding of Vitamin D has been shown altering the cytokine composition. Autoimmune diseases and inflammatory bowel disease have beneficial effects of vitamin D, as well as cardiovascular disease, infectious disease and cancer (28). Vitamin D moreover influences cell proliferation and hormone control.

25-OH-vitamin D is shown to give inhibitory intra- and extracellularly effects on viral load (29). The natural course of HCV infection thereby improves, including reduced level of fibrosis. Adding vitamin D to the treatment of HCV with PEG-IFN/Ribavirin, could improve the virological response (30). The level of vitamin D is inversely correlated with liver disease, it is not found to correlate significantly with the level of liver enzymes as ALT and AST (31).

The population of Tromsø, a city above the Polar circle, has average values of vitamin D lower than the recommended sufficient levels (75 nmol/L). For example, the Tromsø Study demonstrated that vitamin D levels were 64.5 nmol/l ± 22.6 and 58.4 nmol/l ± 20.6, respectively in women and men (32). The situation is even more severe in the youth, when 60,2 % of 15-19 years old healthy students had vitamin D deficiency or insufficiency, which is blood serum 25-OH-vitamin D under 50 nmol/l (33). Out of these 16,5 % were deficient (< 25 nmol/l), and 1,6 % even had a severe deficiency (< 12,5 nmol/l). Girls had significantly higher levels than boys, with means of respectively 54,2 nmol/l ± 23,2 and 40,5 nmol/l ± 20,5.

Work process, materials and methods

Work process

Originally the idea was to perform a study on the quality of life of hepatitis C affected patients, undergoing the treatment at the University Hospital of Northern Norway (UNN) in Tromsø. This was of our particular interest, because of the many known adverse and side effects of the PEG-IFN/Ribavirin treatment, as mentioned in the introduction chapter. This would have been an interventional study.

However, due to additionally more specific and strict rules, regarding the application for the regional ethics committee; it did not allow us to initiate the inclusion process in the given time for this assignment.

As a consequence of that, we decided to concentrate on the description side of the study, and primarily on vitamin D status, genotypes of hepatitis C and IL28B.

The plan was to collect the data from the patients by June 2014. This was available in the journal system of the hospital. From August 2014 up to February 2015, the conductor of this study would be in clinical practice. Thus, this time was planned to be used to find, read and categorize articles relevant for this study. March up to June was planned for writing, finding the last data, calculating the data and putting it all together.

The study was approved by the Norwegian regional ethical committee (REK), with the reference number 2015/608.

Materials

This study is a retrospective cohort study. We have included a total of 107 HCV infected patients, followed up at the department of Gastroenterology, at the UNN. Originally there were 109 patients, but 2 of them demonstrated negative HCV-PCR, and were therefore excluded from the study. The materials were retrospectively collected, from the computer journal system of the UNN. The point of time for the first follow up visit, for each of the patients included, ranged from autumn 2012 up to spring 2015. Autumn 2012, the department of Gastroenterology started up with routine blood samples of vitamin D, for all the HCV

infected patients newly referred to the outpatient clinic. This was why we chose to start our collection of data at this point of time.

Depending on stage of disease, genotype and clinical evaluation, part of the patients were followed up with medical PEG-IFN/Ribavirin treatment, while the rest continued without medical treatment. Some patients would achieve SVR, while others would not achieve SVR (non-SVR), or not finish medical treatment until completion of this study. These four outcomes were included in the study.

All biochemical and clinical tests were originally taken in clinical purpose as a standard of clinical care. The biochemical tests have been analyzed at the Department of Clinical Biochemistry, UNN. The biochemical tests included in this study were: 25-OH-vitamin D, ALT, AST, viral load of HCV, genotype of HCV and genotype of IL28B. These were measured at the first session of follow up at the hospital. Other parameters included were: year of birth, sex, Ishak and HAI score in liver biopsies.

The HCV quantification was performed with a combined COBAS® AmpliPrep and COBAS® TaqMan®, version 2.0 test. The material applied, was 650 µl plasma or serum blood. COBAS® AmpliPrep was used for preparation. This was accomplished by isolating the HCV RNA, consisting of lysis, binding to a known amount of magnetic glass particles and subsequently rinsing. COBAS® TaqMan® was used for amplification and detection of HCV RNA. It performed a 48 times amplification of the material with specific primers. Usage of double fluoroform marked probes, gave rise to real time detection of substrate by the AMBILINK system. The result was presented in international units per milliliter (IU/ml).

HCV genotyping was performed with a combined COBAS® AmpliPrep system and VERSANT® Line Probe Assay (LiPA), version 2.0 test. The latter utilized a reverse hybridization technology to detect genotypes 1-6 of HCV, including subtypes, but excluding genotype 7. The COBAS® AmpliPrep system was used for the preparation. An AutoBlot H 3000 machine completed the final interpretation.

IL28B-genotype was also determined by PCR technique. The 3 different SNPs CC, CT and TT were determined by the adding of probes, fluorescently tagged, targeting either C and/or T, 3 kb upstream at rs12979860 on the IL28B gene.

For measurement of total 25-OH-vitamin D, the laboratory of UNN used a liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique, measuring 25-OH-vitamin D_2 and D_3 each for themselves. The material was 0,8 ml serum blood. It was presented in nanomoles per liter (nmol/l). Minimum analyzed data was 2 nmol/l and 10 nmol/l, for respectively vitamin D_2 and D_3. Normal range was defined in between 50-150 nmol/l, suboptimal levels were under 50 nmol/l, and deficient levels under 25 nmol/l. Because we later discovered that none of the patients had detectable vitamin D_2 levels, we knew nobody used vitamin D supplementation, and vitamin D_3 would equal the total vitamin D level.

The material for measurement of ALAT and ASAT was 240 µl serum blood. It was presented in units per liter (U/l). Normal ranges for ALAT were 10-45 U/l for women, and 10-70 U/l for men. Normal ranges for ASAT were 15-35 U/l for women, and 15-45 U/l for men. The normal ranges increased slightly with age.

Liver biopsies were scored according to the Ishak scales for fibrosis grading and inflammatory activity according to the HAI score at the Department of Pathology at UNN. See tables 3 and 4 in the appendix for closer classification.

Methodes

We did a systematical review of the journals of each of the 109 patients who had been followed up for chronically HCV infection at UNN, dating back from 2012, up to spring 2015. We reclaimed several parameters. These included liver tests, such as ALT and AST, as well as the viral load of HCV. We used a measurement of the total amount of 25-OH-vitamin D, which consists both of 25-OH-vitamin D_2 and 25-OH-vitamin D_3. In addition we reclaimed the genotypes of HCV, the viral load of HCV and the genotypes of ILB28 at rs12979860. We used the Ishak score for grading of liver fibrosis, and the HAI for a combined grading of liver necrosis, inflammation and fibrosis. The data was then plotted in a spreadsheet from IBM's SPSS version number 22. We later used the same program for calculating statistics from the reclaimed data.

All statistical tests were tested for significance at 95 % confidential interval level, with H_0 set to $p < 0,05$. The main purpose with our study was to look for any association between levels of 25-OH-vitamin D and different parameters linked to HCV infection. For dichotomies, we

used independent-sample T-test, for comparisment of two nominal parameters, we used chi-squared test, for grouped variables, we used one-way ANOVA, and for scaled variables, we used Spearmen's correlation. The latter was chosen over Pearson's correlation because of possible single runaways, potentially interfering with the rest of the dataset.

For some of the values we chose to create dummy variables/dichotomies, mainly for increasing the number of persons included in the various groups. We made a dichotomy in the Ischak score, with a cutoff value in between 2 and 3. We did this because the number of patients distributed in the groups over the value 3 were low. We determined Ishak score 1-2 as mild liver disease, and Ishak score 3-6 as more serious liver disease. The first was without hepatic portal bridges, while the latter was with hepatic portal bridges. The same was done with the HAI score, were the cutoff value was set between 9 and 10.

We created two tables. Table 1 gives a demographic presentation of this study, regarding gender distribution. All the parameters which had a normal distribution, are presented with the mean value, plus/minus 1 standard deviation. All the parameters which had not a normal distribution, are presented with the median number, and range as parenthesis. Table 2 presents stratified levels of vitamin D, with regard on viral genotype, IL28B genotype, Ishak dichotomy and HAI dichotomy.

The dates of birth for the patients were available, but not the specific ages at the first follow up session. To estimate the age at the beginning of follow up, we created a new parameter by subtracting 2013 from the year of birth. We did this because we found the highest number of patients treated in the year 2013, and the two surrounding years. This was a simplification, but the amount of patients included made this seem neglectable, or at least satisfactory. We regarded age differences to be more important on an individual level, than on a population level.

Results

Out of 107 patients, 71 (66,4 %) were men, while 36 (33,6 %) were female. The demographics of the population of this study, is presented in table 1.

2 persons missed values of viral load, and were therefore excluded from the final analysis. Viral load was significantly higher in men versus women. 4 persons had 0 IU/ml in viral load of HCV. The maximum value was 26 496 000 IU/ml. 29 persons missed 25-OH-vitamin D values, these were also excluded from the statistical analysis, where vitamin D was a variable of interest. None of the patients had detectable levels of 25-OH-vitamin D_2, making D_3 equaling total vitamin D.

71 persons (65,1 %) were not treated medically for chronically HCV infection. 10 persons (9,2 %) were still under medical treatment at completion of this study. 26 persons (23,9 %) had achieved SVR, while 2 persons (1,8 %) were non-SVR (figure 1). 92,9 % out of those finishing medical treatment, had achieved SVR.

We were able to obtain Ishak score from 38 persons. 18 people (47,4 %) had a score of 1 or 2, while 20 people had a score from 3-6 (52,6 %). We were able to obtain HAI score from 34 persons. 20 persons (58,8 %) had a score under 10, while 14 persons (41,2 %) had a score from 10 and up.

Age and vitamin D were normally distributed, which resulted in skewness of -0,41 and 0,53 respectively, and kurtosis of -0,79 and -0,40 respectively. The variables ALT, AST and viral load were skewed. The statistics were carried out with the use of non-parametric analysis.

There were no significant correlation between 25-OH-vitamin D and viral load or age, in either gender. Nor were there any correlation between 25-OH-vitamin D and ALT or AST, in either gender.

The distribution of vitamin D levels across different groups of genotype and histological classification, is presented in table 2.

Subjects with IL28B genotype TT had significantly higher vitamin D levels, than the two other genotypes. However, while subdividing the genotypes into two groups: CC and others (CT and TT), there were no significantly differences in 25-OH-vitamin D levels.

Discussion

In our study we have presented the description of our hepatitis C virus infected population with the special emphasis on vitamin D status. This has not yet been done in Norway.

Demographically we noticed a majority of male patients, constituting approximately 2/3 of the total amount of patients included in this study. This distribution was similar to the distribution from 1999, also measured in a Northern Norwegian population (2). The overall mean age was now approximately a decade higher than last time. The incidence of new cases of HCV has been shown to go down (3). This might implicate our population consisting of, by a great proportion, the same population as in 1999. Interestingly the male group in our study, both was significantly older, with a mean age of 45,7 years, compared to the females mean of 38,5 years, as well as having significantly higher viral loads. One explanation could be that male patients presumably avoided contact with the health services for a longer period of time, thereby increasing disease development before getting treatment.

The distribution of HCV genotype resembled the measurements from previous descriptions of our population (1). With no occurrences of other genotypes than 1-3, this was also consistent with the assumed global distribution (4).

About 2/3 of the patients followed up, were not treated medically for HCV infection. Reasons for not starting treatment were amongst others ongoing drug abuse and psychiatric comorbities complicating treatment. Many patients wanted to postpone the medical treatment because of the known adverse and side effects of medication with PEG-IFN. Out of the patients finishing treatment, a major part were able to achieve SVR. This result was better than the results described in previous studies (20). This could be explained by a good follow up at the outpatient clinic, and/or a coincidence, due to the relatively few pertinent patients included. With the new spectrum of medical treatments becoming available, such as the DAA's, more of the patients previously not treated, or with non-SVR, could receive treatment (24).

It was of importance to us to describe the levels of vitamin D in relation to HCV, due to its known influence on infections and immunology. The discovery of the vitamin D-receptor (VDR) in leucocytes, presumably makes vitamin D play an important role as an immune system modulator (27). Vitamin D binding to these receptors, has been shown altering

cytokine composition, improving outcome of several diseases (28). In general, levels of vitamin D were inversely correlated with liver disease (31). Relating to HCV infection, higher levels of vitamin D decreased HCV concentration, both intra- and extracellularly (29). This, as well as reducement of liver fibrosis with increased levels of vitamin D, can be considered important factors in improvement of the natural course of HCV infection. Adding vitamin D to the medical treatment of PEG-IFN/Ribavirin, has also been shown to give beneficial effects on treatment outcome (30). This all shows that vitamin D could improve outcome of HCV infection, both naturally and with medical treatment.

Our HCV infected population demonstrated levels of vitamin D under the recommended sufficient levels (75 nmol/l), also having somewhat lower levels of vitamin D than the general Northern Norwegian population. One reason for lower levels of vitamin D was probably caused by the geographical location above the Polar circle, giving a more irregular exposure to sun light throughout the year. This did not explain the noticed difference to the general population. Hepatitis itself has been linked to reduced levels of vitamin D (31). In addition, poor health might lead to less outdoor activities, resulting in reduced sun exposure. The majority of HCV infected people have a history of injecting drug use, as well as poorer socioeconomic status. This might lead to less awareness of the subject, not obtaining supplementation through food or medicine, and a reduced capability of traveling to sunny destinations. We did not find any detectable levels of 25-OH-vitamin D_2, in our reclaimed data. Thereby we could conclude that none of the patients used vitamin D supplementation.

We found no significant associations between levels of vitamin D and the grade of liver fibrosis. This would have suited the theory of VDR binding, reducing activity of hepatic stellate cells, subsequently leading to reduced liver fibrosis (29). However, we found that patients with higher grade of fibrosis had higher levels of 25-OH-vitamin D. Nevertheless this was still not significantly. It is hard to explain why so, and we would have expected the opposite. There are several plausible explanations: First of all there could be a bias in our relatively low number of samples, especially those on Ishak and HAI. In addition, patients with more pronounced liver fibrosis, giving more severe disease, might have had a higher degree of awareness of their disease. Maybe this influenced their lifestyle, by amongst other turning into a healthier diet, consisting of more vitamin D.

Furthermore, in our study, subjects with IL28B genotype CC, also showed no significant differences in levels of vitamin D from the other genotypes. This result may again have been biased due to low number of samples. Likewise vitamin D have been shown to influence and improve cytokine and subsequently viral response, different genotypes of IL28B are also of importance for different outcomes of HCV infection (27). Genotype CC seemed to give the highest rates of SVR and RVR, predominantly for HCV genotype 2 and 3 infected persons (14,15). Maybe vitamin D and genotypes of IL28B would not influence each other, but complementary could improve the outcome of HCV infection.

And finally, HCV infected patients with higher inflammation, demonstrated lower vitamin D levels, but the results did not reach the statistical significance. This also suited the description of vitamin D, not significantly correlating with the liver enzymes, which we had measured in our study, even though vitamin D inversely was found to correlate with liver disease (31).

Our study had some shortcomings. First of all we did not have the data for fibrotic grade and the level of inflammation, in all 107 patients included in our study. The small amount may therefore have influenced the power of our study. Secondly, the 25-OH-vitamin D level is not a constant value, and the levels depend widely from the season when the samples are obtained, as well as the recent nutritional habits. And finally the total amount of subjects included in our retrospective study were also not substantial enough, but we were limited in time for collection and access.

We still feel that our results are valuable as there are no other studies available which describe the vitamin D status in the Northern Norwegian hepatitis C population, and our conclusions have an important outcome for the clinical care practice.

Conclusion

Our HCV infected patients have lower vitamin D status than the general Northern Norwegian population.

There were no significant associations between vitamin D levels and histological grades and the degree of inflammation, however subjects with higher degree of inflammation tended to have lower 25-OH-vitamin D levels.

Measuring of vitamin D status in HCV infected patients is a valuable tool in discovering and prevention of vitamin D deficiency in this fragile patient group, and should be included in the baseline blood test panel at the first visit to the outpatient department.

Further studies and studies with larger amounts of patients and interventional studies are emerged to detect if vitamin D plays an important role in inflammation grade and fibrosis level, in the patients infected with chronic hepatitis C virus.

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Appendix

Figures

Figure 1- Treatment outcome

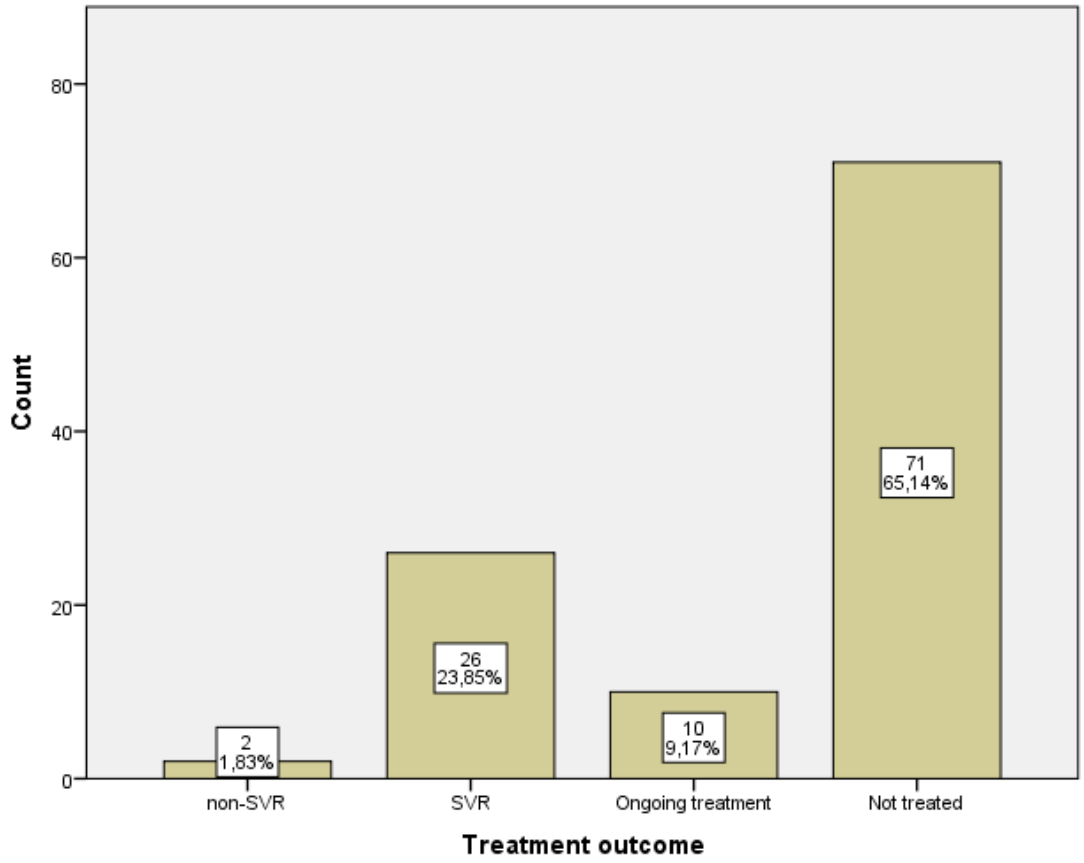

FIGURE 1 - Presenting the frequencies, included in percentage distribution, of different outcomes of treatment. Categories being not treated, ongoing treatment, sustained virological response (SVR) or not achieved SVR (non-SVR)

Tables

Table 1 - Demographics

The demographics of the study population	**Men,** *n = 71*	**Women,** *n = 36*	**P-value**
Age (years)*	45.7 ± 11.0	38.5 ± 11.6	0.003
Genotype HCV, n = 99			
1	24	16	NS
2	8	6	
3	33	12	
Genotype Il28B, n = 79			
CC	24	11	NS
CT/TT	29	15	
ALT (U/l)**	79 (541)	61 (445)	NS
AST (U/l)**	50 (235)	38.5 (229)	NS
Viral load (IU/l)**	2 274 000 (26 495 967)	962 000 (7 715 985)	0.003
Vitamin D (nmol/l)*	53.9 ± 28.2	56.7 ± 5.3	NS

TABLE 1 - Presenting the demographics of the study population. If significant on p < 0,05 level, the p-value is presented. If not significant, it is noted with not significant (NS). *The variables are presented in median (range), due to non-normal distribution. **The variables are presented in means ± SD, because of normal distribution.

Table 2 - Vitamin D levels

Vitamin D levels stratified by viral genotype, Il28B genotype, ISHAK score and HAI				**Significance**
	Genotype HCV			
	1, n = 32	*2, n = 12*	*3, n = 29*	
Vit. D (nmol/l)	57.5 ± 26.9	56.3 ± 27.1	52.2 ± 26.9	NS
	Genotype IL28B			
	CC, n = 27	*CT, n = 32*	*TT, n = 5*	
Vit. D (nmol/l)	52.5 ± 21.4	54.8 ± 30.1	92.4 ± 19.3	0.007
	Ishak			
	1-2, n = 10	*3-6, n = 15*		
Vit. D (nmol/l)	50.0 ± 25.3	56.3 ± 32.2		NS
	HAI			
	1-9, n = 13	*10-18, n = 8*		
Vit. D (nmol/l)	54.4 ± 26.3	48.1 ± 28.4		NS

TABLE 2 - Presenting vitamin D levels within the different subgroups of Genotype HCV, Genotype IL28B, Ishak and HAI. The levels are presented in means ± SD. If significant on $p < 0,05$ level, the p-value is presented. If not significant, it is noted with not significant (NS).

Table 3 - Ishak score

Modified staging: architectural changes, fibrosis and cirrhosis

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging (portal to portal (P-P) as well as portal to central (P-C))	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
Maximum possible score	6

TABLE 3 - Presenting the Ishak scoring criteria, with the maximum possible score being 6.

Table 4 - HAI

Modified Histological activity index grading: necroinflamatory scores

	Score
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around 60% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
B. Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis+occasional portal-central (P-C) bridging	4
Zone 3 necrosis+multiple P-C bridging	5
Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation*	
Absent	0
One focus or less per 10 x objective	1
Two to four foci per 10 x objective	2
Five to ten foci per 10 x objective	3
More than ten foci per 10 x objective	4
D. Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Maximum possible score	18

TABLE 4 - Presenting the Histological activity index (HAI) scoring criteria, with the maximum possible score being 18. *Does not include diffuse sinusoidal infiltration by inflammatory cells.