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Salivary Gland Dysfunction and Its Association with Hepatitis C Infection

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Salivary Gland Dysfunction and Its Association with Hepatitis C Infection

by

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THESIS

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In loving memory of my beloved grandmother

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Abstract

Salivary Gland Dysfunction and Its Association with Hepatitis C Infection

Chelsia Qiuxia Sim

We hypothesized that there was a difference in the unstimulated whole salivary flow rate (UWSR) in chronic HCV subjects compared to those with chronic liver disease (CLD) due to other causes.

Purposes of this study:

1. To compare UWSR and the prevalence of salivary hypofunction (UWSR<0.1mL/min) in chronic HCV and non-HCV subjects with CLD.
2. To examine possible factors that may affect UWSR in subjects with CLD.
3. To compare the correlation between UWSR and patient's subjective assessment of oral dryness, oral discomfort, difficulty in swallowing dry food and any food without additional liquids, difficulty in speaking and lip dryness, in patients with CLD.

Methods: A case-control study examined 76 chronic HCV and 52 non-HCV subjects from a tertiary-referral liver clinic (n=128). None had known predisposing factors for salivary hypofunction. UWSR was measured and a 6-item visual analog scale (VAS) questionnaire was used to assess subjective symptoms of salivary gland dysfunction. An oral examination was done to identify lichenoid lesions. The Student's t-test and Pearson's correlation tests were used to compare groups and linear regression was used to identify predictors of salivary flow. **Results:** Mean age, proportion of male subjects and proportion with cirrhosis were comparable in chronic HCV and non-HCV groups. Mean UWSR (mL/min±S.D) and prevalence of salivary hypofunction were lower in the HCV versus non-HCV group (0.26±0.15 versus 0.30±0.21, p=0.22 and 10% versus 17%, p = 0.17). HCV status was associated with lower UWSR but in multivariate analysis only female gender, use of anticholinergic medication and presence of cirrhosis were statistically significant predictors of UWSR. All VAS scores were significantly higher in the HCV than non-HCV group ($p<0.05$). A moderately strong correlation between UWSR and VAS scores was shown amongst HCV subjects (r-values -0.45 to -0.30). **Conclusions:** Reduced salivary flow is frequent in CLD patients and associated with cirrhosis, using anticholinergic medication and being female. Amongst patients with HCV, the moderately strong correlation between UWSR and VAS scores suggest the VAS questionnaire maybe a useful tool to screen for salivary hypofunction and lead to early implementation of preventive measures to avoid dental complications.

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(1) Introduction

My study goals are to determine whether salivary function is different in patients with chronic hepatitis C virus (HCV) infection compared to patients with chronic liver disease from other causes. The specific study objectives are:

1. To compare unstimulated whole salivary flow rate (UWSR) in chronic HCV infected and non-HCV infected patients with chronic liver disease.
2. To determine the prevalence of salivary hypofunction, defined as UWSR < 0.1 mL/min, between chronic HCV infected and non-HCV infected patients with chronic liver disease.
3. To examine possible factors that may affect the UWSR in patients with chronic liver disease.
4. To correlate UWSR and patient's subjective assessment of oral dryness, oral discomfort, difficulty in swallowing dry food and any food without additional liquids, difficulty in speaking and lip dryness, in chronic HCV infected and non-HCV infected patients with chronic liver disease.
5. To determine the prevalence of oral lichenoid lesions in chronic HCV infected and non-HCV infected patients with chronic liver disease.

(2) Background and Significance

(i) Hepatitis C virus infection

Hepatitis C virus (HCV) is a small, enveloped, single-stranded, positive sense RNA virus. It was first discovered in 1989 and has been classified within the

Flaviviridae family as a separate genus.[1] The overall worldwide estimated prevalence of HCV infection is about 2-3%, with approximately 120-180 million anti-HCV-positive people, most of whom are chronically infected. The global prevalence of HCV infection varies greatly. In Europe, the overall prevalence is 1% with a north-south gradient, ranging from 0.5% in the Northern countries to 2% in Mediterranean countries. In Eastern Europe, the prevalence ranges from 0.7% to 5%.[2] In the United States, the National Health and Nutrition Examination Survey (NHANES) found that about 1.6% of the population had been infected with HCV, corresponding to 4.1 million individuals. Amongst these, almost 80% were chronically infected.[3] HCV infection is the most common blood-borne infection in the United States.[4] It is also the most frequent cause of chronic liver disease and the leading cause of death from liver disease in the United States.[5] The principal means of HCV transmission is parenteral, through contact with contaminated blood or blood products. Increased risk of HCV acquisition occurs in those with a history of injection drug use and persons who received blood/ blood component transfusion or organ transplant before 1992. Before 1992, no screening was available for HCV antibodies in donor blood or organs. Since the advent of screening of all blood donations for HCV, the risk of HCV transmission through transfusion in developed countries has declined.[10, 11] Other potential sources of HCV transmission include exposure to an infected sexual partner or multiple sexual partners, [6-8] exposure to infected blood as health care workers, vertical transmission from infected mother to child, tattooing and the use of non-injecting drugs such as intranasal cocaine.[9] Recognized iatrogenic routes of HCV

transmission include organ,[12] tissue[13] and bone marrow [14] transplantation from infected donors, plasmapheresis,[14] hemodialysis and at times during endoscopy[15, 16] if proper sterilization of medical instruments are not carried out.

Upon acquisition of HCV, acute symptomatic hepatitis may occur in a small percentage of patients within 6-12 weeks, but 55-85% of exposed individuals will go on to develop chronic HCV infection.[17, 18] Amongst these, 5% to 20% are reported to develop cirrhosis in the next 20-25 years. Cirrhosis predisposes patients to hepatocellular carcinoma, with a risk of 1-11% per year.[19]

A classification of HCV into different genotypes has been developed based upon nucleotide sequencing of many isolates collected worldwide. HCV is classified into six genotypes, labeled 1 to 6. In addition, as a result of frequent error in its course of replication and lack of repair mechanisms, within a single infected individual, the initial inoculum of HCV can evolve into a swarm of closely related viral species called quasispecies.[20] There is a large worldwide variation in the distribution of the different genotypes in the HCV infected population. The most common HCV genotype in Europe, the US and Japan is genotype 1 while genotype 3 is most frequently found in Thailand, Singapore and parts of India. In the Middle East, Egypt and Central Africa, genotype 4 is commonly encountered whereas genotypes 5 and 6 are mostly present in South Africa and Southeast Asia.[21]

Recent epidemiological studies showed that there has been a decrease in the incidence of HCV infection partly due to the screening of blood/organ donors for HCV and education programs targeted at reducing the spread of human immunodeficiency virus (HIV), which has shared risk factors with HCV. However, despite the decrease in incidence of HCV infection, there is an expected increase in the prevalence of advanced liver disease due to HCV infection due to the delayed clinical manifestation of chronic HCV infection. Hence, it is important for health care professionals to have a better understanding of the oral complications of HCV infection and chronic liver disease in order to play a role in optimizing the management of these patients.

(ii) Extrahepatic manifestations of Hepatitis C infection

HCV is a hepatotropic virus that not only causes damage to hepatocytes, but also causes various extrahepatic manifestations. There are some evidence showing the presence of HCV negative strand RNA consistent with active HCV replication in the plasma,[22] peripheral blood mononuclear cells,[23] in sperm of HCV-infected patients[24] and malignant lymphoma tissue of the parotid gland[25]. The most well-documented extrahepatic manifestations of HCV infection are essential mixed cryoglobulinemia and porphyria cutanea tarda.[26, 27] Essential mixed cryoglobinemia is a multisystem disorder, characterized clinically by purpura, weakness, arthralgia and complications of cryoglobulinemia includes systemic vasculitis and membranoproliferative glomerulonephritis.[28] Serologically, it is characterized by presence of temperature-sensitive proteins called cryoglobulins.

Mixed cryoglobulinemia can occur in approximately 36-54% of chronic HCV infected patients but it is symptomatic in a smaller proportion of patients.[29, 30] Evolution from cryoglobulinemia into B-cell lymphoma has been reported in 5-8% of cases. It was postulated that HCV could infect lymphoid tissue in patients with mixed cryoglobinemia, indicating a direct role of HCV in the induction of disease.[31] Another common extrahepatic manifestation of HCV infection is porphyria cutanea tarda, a condition resulting from a reduction in hepatic uroporphyrinogen decarboxylase activity. The characteristic clinical features include increased skin fragility and cutaneous lesions such as erythema, blistering, scarring and pigmentation on exposure to sunlight. The exact mechanism of association between chronic HCV infection and porphyria cutanea tarda is unknown. In addition to these systemic manifestations, there are oral manifestations of HCV infection.

(iii) Oral Manifestations of Hepatitis C Infection

Since the discovery of HCV, several oral manifestations have been linked with HCV infection, including oral lichen planus, HCV- associated sialadenitis and HCV has been hypothesized to be a viral etiological agent for Sjögren's syndrome.

Oral lichen planus (OLP) is a chronic immunological mucocutaneous inflammatory condition. It has various clinical appearances, mainly characterized as reticular form, atrophic/erythematous form or erosive form.[32] The mean age of

onset is around the fifth decade with female predilection. The condition usually involves multiple sites in the oral cavity with the buccal mucosa being the most common site of occurrence, with bilateral involvement.[33-35] Association between OLP and liver disease was first reported by Rebora et al in 1978 and subsequent studies showed that anti-HCV antibodies were found up to 23% of patients with OLP,[36-38] although there is great variation amongst different geographical regions.[39-43] Details regarding the mechanisms of HCV causing OLP remains unclear and the association between HCV infection and OLP is controversial. Some dentists recommend testing for anti-HCV antibodies in patients with OLP, although it is recognized that more studies need to be done to determine the association between the two.[43, 44]

Sjögren's syndrome is a chronic, autoimmune, inflammatory disorder, which mainly affects the exocrine glands. Clinically, it manifests as complaint of persistent dry eyes (keratoconjuctivitis sicca) and dry mouth. Histopathologically, it is characterized by focal infiltration of CD4+ T cells into the salivary and lacrimal glands.[45, 46] Sjögren's syndrome can present alone as primary Sjögren's syndrome or in association with another autoimmune disease (such as rheumatoid arthritis or systemic lupus erythematosus) as secondary Sjögren's syndrome. The focal lymphocytic infiltration in the exocrine glands can lead to focal sialadenitis in the salivary glands.[47] After the initial discovery of HCV, some authors found an association between HCV with Sjögren's syndrome. A possible relationship between Sjögren's syndrome and HCV was first postulated in 1992 by Haddad et

al,[48] who noted the presence of characteristic Sjögren's syndrome histological changes in the salivary glands of patients with HCV infection. Several mechanisms have been postulated for the pathogenesis of Sjögren's syndrome associated with HCV infection, including a direct infection and proliferation of HCV in salivary glands, molecular mimicry of HCV and salivary glands and/or formation of immune complexes containing HCV triggering an immune – mediated injury.[49] Pawlotsky et al suggested that HCV-related Sjögren's syndrome is a result of replication of HCV in salivary gland tissues or as result of an immunological process triggered by HCV infection.[29] It has been proposed by Ramos-Casals et al that HCV is a sialotropic virus, but the role of HCV in the pathogenensis of Sjögren's syndrome is still unknown.[50, 51] Several studies showed that salivary hypofunction is present in chronic HCV infected patients.[52-54] Chronic HCV infection may mimic the clinical, histological and immunological features of primary Sjögren's syndrome, although patients with HCV related Sjögren's syndrome are usually older, have lower prevalence of anti-SSA/Ro and anti-SSB/La and parotid swelling.[51] Many studies have examined the association between Sjögren's syndrome and HCV and the controversy regarding the role of HCV in the etiopathogenesis of Sjögren's syndrome led to the decision to include the presence of HCV infection, amongst other medical co-morbidities, to be consider an exclusion criterion for the diagnosis of Sjögren's syndrome in the 2002 American- European Consensus Criterion of Sjögren's syndrome.[55]

With the exclusion of HCV infection for the diagnosis of Sjögren's syndrome, the term HCV-associated sialadenitis was coined to describe the salivary tissue changes noted in HCV infected patients to differentiate the condition from Sjögren's syndrome. Pirisi et al reported that in their Italian population, about 78% of HCV infected patients had mild sialadenitis.[56] Presence of sialadenitis in HCV infected patients could lead to salivary hypofunction.[57, 58] This led to several other studies that examined the prevalence of salivary hypofunction in chronic HCV infected patients.[39, 52, 59] The reported prevalence of salivary hypofunction in HCV infected population varies widely depending on geographical regions, from 17% to 62% and the presence of sialadenitis varies from 11% to 47% (Table 1).[48, 52, 59, 60] Notably, there are limited data on the prevalence of objective measurement of dry mouth and dry eyes in patients with chronic liver disease, especially those with chronic HCV infection.[61] In terms of the possible pathogenic mechanisms, it was postulated that HCV could have caused salivary gland injury, either by a direct or indirect mechanism. HCV RNA has been isolated from saliva and salivary glands of patients with HCV-associated sialadenitis, [62-64] suggesting several possible mechanisms for HCV affecting salivary gland function. These included a possible direct viral effect on salivary gland tissue, a virus-induced immune reaction leading to an inflammatory reaction or accumulation of immune complexes in the salivary gland tissue. Others argued that the presence of HCV RNA in saliva could be due to blood contamination during saliva collection. [65] On the other hand, studies had suggested that the association between HCV infection and HCV-associated sialadenitis is indirect, most likely due to cryoglobulinemia,

leading to immune complexes being deposited within the salivary gland tissue, causing lymphocytic infiltration from the circulation and the subsequent development of sialadenitis. [66, 67]

The published data evaluating the association between HCV and salivary function were limited. Prior studies were done before the 2002 American-European Consensus Criterion for Sjögren's syndrome, in which authors were examining either (i) the prevalence of HCV infection in patients with Sjögren's syndrome, or (ii) the prevalence of Sjögren's syndrome in chronic HCV infected patients to determine the association of HCV infection and salivary gland dysfunction and sialadenitis. Some of these prior studies had no control groups[52, 53] while others did not use standardized methods for the measurement of salivary function.[39] To overcome some of these limitations, my study aims to examine salivary gland dysfunction and its the association with chronic liver disease, especially chronic HCV infection, using a standardized method for the collection of unstimulated whole saliva to measure salivary function and excluded patients with Sjögren's syndrome.

(iv) Salivary Hypofunction and Its Dental Implications

Saliva is produced by 3 pairs of major salivary glands, the parotid, submandibular and sublingual glands, and hundreds of minor salivary glands in the oral cavity. Saliva bathes teeth and oral mucosa, playing an important role in maintaining oral health. Reduction in the amount of saliva produced throughout the day (salivary

hypofunction) can lead to deterioration of dental health and significantly affect quality of life. An objective assessment of salivary gland function can be achieved by measuring the salivary flow rate. This can either be unstimulated or stimulated, whole or individual major salivary gland function. Unstimulated whole saliva is comprised of secretions produced by the major and the minor salivary glands and these secretions are produced in the absence of exogenous stimuli. This is unlike stimulated whole salivary, in which secretions are produced in response to gustatory or masticatory stimuli. Studies have been performed in healthy individuals and the normal flow rate of unstimulated whole saliva (UWSR) was determined to be 0.3-0.4 mL/min,[71] while that of stimulated whole saliva (SWSR) was about 1.5-2 mL/min[72] hence showing that there is range for reported normal valuses for both UWSR and SWSR. Hence, it is difficult to determine whether an individual has an abnormal salivary flow rate but it is generally accepted that salivary hypofunction is present when UWSR < 0.1 mL/min or SWSR < 0.5mL/min.[69] Unlike objective measurement of salivary flow rates, xerostomia is defined as a subjective feeling of oral dryness, which may or may not be accompanied by an objective reduction in the secretion of whole saliva. [69] There are data to suggest that a 50% reduction of salivary flow rate within an individual would lead to complaints of xerostomia which explains why an individual may complain of oral dryness yet not meet the cut-off point as mentioned above and xerostomia may not be accompanied by reduced salivary flow rates at all times. [71] Conversely, the absence of xerostomia does not necessarily mean that there is adequate salivary secretion. [68] In addition, oral comfort and the feeling of oral

dryness is more dependent on UWSR as compared to SWSR since only a small percentage of time during the day is spent eating.[72] In my study, unstimulated whole salivary flow rate was used because many studies have shown that unstimulated salivary flow rate was more important than stimulated salivary flow as a determinant for oral dryness, [68, 69, 73] and has been shown by Gotoh et al that there is a lack of consistency in the repetitive collection of stimulated whole saliva, making measurement of SWSR is less reliable than UWSR.[74]

Numerous well-established causes of salivary gland dysfunction are recognized including radiation therapy to the head and neck region, use of medications (anticholinergic agents), salivary gland infection or autoimmune exocrinopathies (Sjögren's syndrome). Other potential factors affecting salivary gland function are dehydration, nutritional deficiencies, psychological disorders, oral sensory dysfunction and cognitive/neurological alterations.[75]

Salivary hypofunction has dental implications such as an increased risk of developing dental caries at the cervical and/or incisal surfaces or cusp tips, mucosal infections such as oral candidiasis, burning sensation on tongue, depapillation of tongue, dysphagia and dysgeusia.[72, 76-78] Management of salivary hypofunction should be implemented once the diagnosis of salivary hypofunction is made. This involves alleviating symptoms of oral dryness, implementation of preventive measures, treating any pathologic oral conditions and if possible, treating the underlying cause of salivary hypofunction.[76, 78, 79] To alleviate the symptoms of dry mouth, several palliative measures can be taken. Salivary

substitutes and lubricants with moistening properties can be introduced to aid in lubrication and hydration of the oral tissues and maintaining oral health and function. Use of sugar-free gum or candies to stimulate saliva flow and frequent sips of water can also provide transient relief of symptoms of dry mouth. As saliva acts as a buffer and has protective mechanisms against dental caries, preventive measures should be started to prevent development of dental caries. Regular dental checkups with annual radiographs can identify early demineralization of dental hard tissues. In addition, meticulous oral hygiene, a low sugar diet and regular use of topical fluoride are recommended. Prescription of daily topical fluoride plays a vital role in preventing the development of dental caries, especially cervical and smooth surface caries. Topical fluoride can be applied with either a toothbrush or a custom made tray to prevent tooth decay. Use of parasympathomimetic agents to increase salivary production can also be used. This can help relieve symptoms of dry mouth.[78] The clinical goal of my study was to determine whether patients with chronic liver disease, especially those with chronic HCV infection, represent an "at risk" group for salivary hypofunction and utilize this information to make recommendations for implementation of preventive measures to avoid development of dental complications in these patients.

(3) Specific aims and hypothesis

In this study, it is hypothesized that there was a difference in the unstimulated whole salivary flow rate (UWSR) and prevalence of salivary hypofunction in chronic HCV infected patients compared to those with chronic liver disease due to other causes.

The purposes of this study:

1. To compare unstimulated whole salivary flow rate (UWSR) in chronic HCV infected and non-HCV infected patients with chronic liver disease.
2. To determine the prevalence of salivary hypofunction in chronic HCV infected and non-HCV infected patients with chronic liver disease.
3. To examine possible factors that may affect the UWSR in patients with chronic liver disease.
4. To correlate UWSR and patient's subjective assessment of oral dryness, oral discomfort, difficulty in swallowing dry food and any food without additional liquids, difficulty in speaking and lip dryness, in chronic HCV infected and non-HCV infected patients with chronic liver disease.
5. To determine the prevalence of oral lichenoid lesions in chronic HCV infected and non-HCV infected patients with chronic liver disease.

This study was initiated as a result of a clinical observation of multiple dry mouth complaints and increased dental needs amongst chronic HCV infected patients. The majority of prior studies examining HCV-associated sialadenitis were done in

countries outside the United States. Those studies either looked at subjects with Sjögren's syndrome to determine prevalence of HCV or in chronic HCV infected subjects to determine the prevalence of Sjögren's syndrome. No studies were done in the United States, in patients with chronic liver disease to evaluate their salivary function. This study will examine the salivary function of patients with chronic liver disease, focusing on the comparison between those with HCV versus without HCV.

(4) Methods

(i) Study Population

A prospective study was carried out, enrolling subjects from the University of California, San Francisco Liver clinic, a tertiary referral center. One hundred twenty-eight consecutive subjects were consented for the study from November 1st, 2008 through January 2010.

Inclusion criteria for cases:

- Positive HCV status without co-infection with Hepatitis B
- HCV status was confirmed with the presence of anti-HCV antibodies using HCV enzyme immunoassay (EIA) (EIA 2.0 Abbott Laboratories, Abbott Park, IL) and HCV RNA, using polymerase chain reaction (PCR) based assay or the transcription-mediated amplication (TMA) assay
- Not on anti-HCV therapy

Inclusion criteria for controls:

- Presence of chronic liver disease with absence of anti-HCV antibodies and HCV RNA

Exclusion criteria for all study subjects:

- Previous history of head and neck radiation therapy
- Known Sjögren's syndrome
- Previous history of lymphoma
- HIV infection
- Previous history of organ transplant
- Previous history of salivary gland pathology
- Known autoimmune conditions such as autoimmune thyroiditis, rheumatoid arthritits and autoimmune liver diseases such as autoimmune hepatitis, primary biliary cirrhosis.

Information on the subject's smoking history, alcohol and marijuana use and current medication use were obtained using a written standardized questionnaire completed by the subjects, while patient demographics and information on HCV genotype, viral load and severity of liver disease (staging of hepatic fibrosis) were collected from subject's medical record.

Approval from the Clinical Human Research Committee, University of California, San Francisco was obtained before enrolment of study subjects.

Informed consent from all eligible subjects was obtained before any study procedures were undertaken.

(ii) Study Procedures

All study procedures were carried out in a private consultation room, in the UCSF Liver clinic between 9am to 2pm by a single examiner.

a) Unstimulated whole saliva collection

Unstimulated whole salivary flow rate (UWSR) was collected using the spit technique. [80, 99] Before the start of collection, the time of the procedure was recorded, using a 24-hour clock (00:00-23:59). Each participant had not eaten or drank for 60 minutes prior to the collection procedure. If the participant had eaten or drank within the 60-minute period, he/she waited the required amount of time before starting the unstimulated whole saliva collection procedure. For some, if severe oral dryness was present, they were allowed to moisten their mouth with water as long as they do not swallow the water and use just enough water to coat the oral mucosa.

Unstimulated whole saliva was collected into a pre-weighed 50mL conical tubes. The 50mL tube was weighed before and after the collection using a single calibrated electric weighing scale, and the pre- and post- collection weight recorded in the salivary assessment form. During the collection procedure, each participant was seated upright with eyes open and head tilted slightly forward. The participant was instructed to minimize oro-facial movements to minimize

influence on salivary flow, not to swallow and speak during the collection process.

To determine the volume of the saliva from the weight of the saliva, it is assumed for the purpose of the study that saliva was similar gravimetrically to water where 1 gram of water at $4^{o}C$ is equivalent to 1 milliliter (mL) of saliva.

b) Oral Examination

After the unstimulated whole saliva was collected, an oral examination was performed.

The oral mucosal examination was conducted using portable light, disposable tongue depressor and cotton gauze. The oral examination protocol followed the recommended protocol by the World Health Organization, and took no longer than 5 minutes. The procedure included:

a. Examination of lips, particularly looking for cracks, fissures or erythema at the commissures by pulling the lower and upper lip to examine the lower and upper labial mucosa
b. Examination of the buccal mucosa, hard and soft palate, gingiva
c. Examination of dorsum and ventral surface of the tongue, lateral and posterior borders of the tongue
d. Examination of the floor of the mouth by having the patient place the tip of the tongue to the posterior roof of the mouth

Clinical presence/absence of lichenoid lesions and oral candidiasis on the various oral sites was recorded on a standardized oral assessment form.

- Lichenoid lesions were defined as either reticular-like, atrophic and/or erosive. The definitions were as follow:
    1. Reticular form: white keratotic striae with a radiating pattern
    2. Atrophic form: red lesion without ulcerations/erosions with surrounding white striations
    3. Erosive form: shallow ulcerations/erosions with ill-defined margins with surrounding white striations
- For the clinical diagnosis of oral candidiasis, the definition and diagnostic criteria developed for the USA Oral AIDS Collaborative Group and EC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Centre on Oral Manifestations of the Immunodeficiency Virus[81] for the presumptive diagnosis of oral candidiasis was adapted. The definitions are as follows:
    1. Pseudomembranous candidiasis: white maculo-papular plaques that maybe located in any part of the oral cavity and can be wiped off revealing an erythematous surface
    2. Erythematous candidiasis: red areas/plaques usually located on the palate or dorsum of the tongue, with occasional occurrence on the buccal and labial mucosa
    3. Angular cheilitis: red fissures or cracks at the commissure of the lips

c) 6-item Visual Analog Scale Questionnaire

Upon completion of the unstimulated whole saliva collection and oral examination, a 6-item questionnaire, using a visual analog scale (VAS), adapted from Ship et al, was administered to the participant (see appendix). The VAS questionnaire provided subjective assessment of salivary gland dysfunction including degree of dryness of the oral cavity, oral discomfort, difficulty in swallowing dry food and any food, difficulty in speaking due to oral dryness and dryness of the lip.[82] Each VAS item has a score ranging from 0 to 100, with 0 indicating no symptoms and 100 with the most severe symptoms. For analytic purposes, the presence of xerostomia was defined as a VAS score for oral dryness, set arbitrarily to be more than 80. Subjects reporting of VAS > 80 were considered to have xerostomia.

(iii) Statistical analysis

a) Sample size calculation:

To determine the number of subjects in each group needed to detect a difference in mean UWSR. We used the following assumptions: [83]

1. The UWSR from a study by Navazesh et al 1996 that was reflective of normal UWSR.
2. The mean UWSR of subjects with chronic liver disease due to other causes other than HCV infection was similar to normal healthy individuals
3. The standard deviation of mean UWSR was 0.304 mL/min

Assuming there was a difference in mean UWSR of 0.130 mL/min between the two groups (HCV and non-HCV), at least 43 subjects in each group were needed, for a two-sided alpha of 0.05 and a beta of 0.2.

b) Data Analysis:

Prior to data analysis, I checked the normality of the variables to determine the appropriateness for using parametric or non-parametric tests. Knowing the variable analyses were approximately normally distributed, analysis of continuous variables such UWSR, VAS scores between the two groups was done using the unpaired Student's t-test. For comparison of categorical variables, the chi-squared and Fisher's exact tests were performed. For assessment of the correlation of UWSR and patient's subjective assessment of salivary dysfunction, the Pearson's correlation test was used. For the evaluation of the independent predictors of salivary flow, multivariate linear regression was used, controlling for potential confounders such as use of xerostomia-inducing medications, age, gender, viral load and genotype, use of tobacco and alcohol. All statistical tests were two-sided and results with $p<0.05$ are considered statistically significant.

(5) Results

(i) Study Population (Table 2)

A total of 128 subjects, 76 HCV and 52 non-HCV subjects were enrolled between November 1st 2008 through January 30th 2010. Within the non-HCV group, 31 had hepatitis B infection, 14 had non-alcoholic fatty liver disease, 1 had congenital hepatic fibrosis and 6 had cryptogenic cirrhosis. In the HCV group, 47(61.7%) were infected with viral genotype 1, viral genotype 2 and 3 were present in 9 (11.9%), 10 (13.2%) had unknown viral genotype and 1 (1.3%) was infected with viral genotype 5/6. The baseline characteristics of the HCV and non-HCV subjects are shown in Table 2. The mean age of subjects in the HCV and non-HCV groups were similar. The proportion of HCV and non-HCV subjects with liver cirrhosis was comparable. However, there were fewer males and non-Whites in the HCV group compared to the non-HCV group. In both groups, the number of subjects taking medication (any type) were alike, however there is a higher percentage of HCV subjects on anticholinergic medication compared to the non-HCV subjects.

(ii) Unstimulated Whole Salivary Flow Rates (Table 3, 4 and 5)

The mean UWSR (mL/min ± S.D) in the HCV group was 0.26 ± 0.15 mL/min (range 0.01 – 0.63) and median UWSR of 0.25 mL/min. The mean UWSR

(mL/min±S.D) in the non-HCV group was 0.32 ± 0.21 mL/min (range 0.04 – 0.82) and median UWSR of 0.27 mL/min. While the mean UWSR in HCV subjects were numerically lower than the non-HCV subjects, the difference was not statistically significant (p=0.223). The mean UWSR in cirrhotic HCV subjects was lower than in non-cirrhotic HCV subjects (0.30 ± 0.16 vs 0.24 ± 0.13 mL/min, with p value approaching statistical significance, $P = 0.08$).

In subjects taking anticholinergic medication, the mean UWSR was lower than in those not taking anticholinergic medication and the difference in the mean UWSR was statistically significant (p=0.028). The prevalence of salivary hypofunction, defined by UWSR < 0.1 mL/min, was higher in the non-HCV group, although not statistically significant (p=0.174).

When evaluated in the univariate regression model, HCV was not associated with mean UWSR, but gender and use of anticholinergic medication were significantly associated with the mean UWSR (p = 0.012 and p = 0.009, respectively) and the presence of cirrhosis approached statistical significance with p = 0.08. Even after controlling for possible confounding effects of gender and anticholinergic medication use in the multivariate linear regression model, HCV status remained unassociated with mean UWSR. Male gender, presence of cirrhosis and the use of anticholinergic medications were determined to be independent and statistically significant predictors of UWSR.

(iii) Xerostomia and Visual Analog Scales (VAS) (Table 7, 8 and 9, Figure 1-12)

The mean VAS scores, for all 6 items (oral dryness, oral discomfort, difficulty in swallowing dry food and any food, difficulty in speaking and lip dryness), were statistically different between the HCV and non-HCV groups (all $p<0.001$) with significantly higher scores in the HCV than non-HCV groups (all $p<0.001$). The correlation between VAS scores and UWSR showed r-values ranging from -0.45 to -0.30 (all $p < 0.007$) in the HCV group, with oral dryness having the strongest correlation with low UWSR ($r=-0.448$, $p<0.001$). Using the arbitrary cutoff point of VAS > 80 for oral dryness to define the presence of xerostomia, a total of five subjects (4%) had xerostomia (1 in the non-HCV group versus 4 in the HCV group). The mean UWSR was significantly lower in the xerostomia versus the non-xerostomia group ($p=0.01$).

(iv) Oral Examination Findings (Table 10)

In the HCV group, six out of 76 (9%) subjects had some form of lichenoid changes in the oral cavity. Five had white, reticular changes on bilateral buccal mucosa and amongst these five, only one had white reticular changes on the maxillary and mandibular gingiva and bilateral buccal mucosa. The remaining one subject had erosive lesions with white reticular changes on bilateral buccal mucosa and the hard palate. Within the non-HCV group, only 1 out of 52 (2%) subjects had an erosive lesion on the right lateral border of the tongue. There was

no statistically significant difference in the prevalence of lichenoid changes between HCV and non-HCV groups (p=0.24).

(6) Discussion

For more than a decade, many groups have investigated the possible association between HCV infection and salivary hypofunction. [52, 53, 59, 60] The results of this study, which involves patients with chronic liver disease, showed that there was no statistically significant difference in the mean UWSR or prevalence of salivary hypofunction in the HCV and non-HCV group. Therefore, in contrast to prior studies, [53, 59] an association between HCV infection and salivary hypofunction could not be confirmed. Instead, my study detected a lower prevalence of salivary hypofunction in the HCV group compared to the non-HCV group. One possible explanation for this unexpected finding may be that the UWSR in my study population was on the higher end of the normal range of salivary flow rates [71, 84] and hence using a cut-off value of <0.1mL/min underestimated the true prevalence of salivary hypofunction in my study population. Additionally, since the mean UWSR in the HCV group was lower than the non-HCV group, although not statistically significant, it raises the possibility that my sample size was insufficient to detect a true difference between the two groups.

The lack of association between HCV and salivary hypofunction maybe there are racial differences in salivary flow rates and that the racial diversity in my cases

and controls compared to subjects in previously conducted studies limited detection of salivary hypofunction. In my study, subjects were enrolled from a tertiary-referral center in San Francisco, a city with great racial-ethnic diversity. In some of the previous studies, subjects were enrolled from suburban areas in countries like Sweden and Japan with more homogeneous group. No studies have investigated the effect of race on salivary function thus it is hard to predict how may affect the salivary flow rates. Further studies maybe needed to determine if race or ethnicity salivary flow rates.

The mean UWSR in females was lower than males in this study population ($p=0.01$). This is consistent with reported values by several prior studies in healthy subjects that females have lower salivary flow rates compared to males.[59, 70, 85] Although it has been shown in many studies that the UWSR and SWSR in females tends to be lower compared to males, especially in older population, [86] there is no clear explanation for this reported findings. However, it has been speculated that the gender difference is likely due to hormonal fluctuations during puberty, menstruation and pregnancy.[87] Likewise, salivary hypofunction may occur after menopause and it has been shown by studies examining salivary flow rates with use of hormone replacement therapy. [88, 89]

It is well recognized that salivary hypofunction is affected by the use of anticholinergic medications. Ideally, all subjects on anticholinergic medications should be excluded in a study evaluating salivary function since different

anticholinergic medications have different potency, causing varying degree of salivary hypofunction. However, it is impractical to exclude subjects on anticholinergic medications because most subjects with chronic liver disease are chronically ill and are on some form of medication. Hence, while performing the study, I obtained detailed information on the type of medications used by the subjects and during statistical analysis, adjusted for use of anticholinergic medications in multivariate analysis.

A novel finding from the study was the association between the severity of liver disease and salivary function. I found that the presence of cirrhosis was an independent predictor of salivary hypofunction in patients with chronic liver disease. Cirrhosis is the most severe form of hepatic fibrosis and it is a surrogate for the duration of liver disease. It is reasonable to postulate that the prolonged period of liver disease, especially in those infected with HCV, leads to chronic inflammation within the salivary glands accounting for the observation of lower salivary flow rates in HCV subjects. Apart from studies involving salivary function and HCV infection, other studies in patients with other causes of cirrhosis showed salivary tissue changes due to parotid gland swelling in chronic alcohol users with hepatic cirrhosis.[90] Development of autonomic neuropathy has been reported in alcoholic cirrhosis subjects. Presence of cirrhosis, regardless of the underlying cause, can cause autonomic neuropathy and the severity increases with the extent of liver disease, suggesting that liver damage is likely responsible for the neurological deficit.[91, 92] However, in the context of alcoholic cirrhosis,

other factors had been postulated to be related to the salivary gland changes such as nutritional deficiencies and metabolic derangement. Hence, further studies can be performed to understand the association between hepatic cirrhosis and salivary gland function. In my study, there was a larger proportion of subjects with cirrhosis compared to those in prior studies, [59] as a result of performing the study in a tertiary-referral center, and I recognize that this may result in selection bias. Nonetheless, all stages of chronic liver disease were represented in my study, and the inclusion of a substantial number of subjects with cirrhosis allowed me to detect an association between cirrhosis and salivary hypofunction that was previously unrecognized.

The VAS questionnaire was utilized to assess the subjective assessment of xerostomia in patients with chronic liver disease.[82] This has not been used in previous studies assessing xerostomia in HCV population. When assessing the subject's perception of xerostomia, there was significant difference in the severity of patient's subjective assessment of oral dryness, oral discomfort, lip dryness, difficulty in swallowing dry and any foods and difficulty in speaking between the HCV group compared to the non-HCV group. A moderately strong negative correlation exist between the VAS scores for oral dryness, oral discomfort, difficulty in swallowing dry food and any food, difficulty in speaking and lip dryness and the mean UWSR within the HCV subjects, but not in the non-HCV group. These results suggest the 6-item VAS questionnaire maybe a useful tool for health care providers to screen for salivary hypofunction in subjects with

chronic HCV infection. Prevalence of xerostomia was determined from the VAS score for oral dryness. This has never been attempted in prior studies of salivary function. For analysis, we used an arbitrary cutoff point of VAS > 80 (scale 0 to 100), yielding a 4% prevalence of xerosomia. This prevalence is low compared to other studies which reported rates of xerostomia varying from 11% to 22%.[93, 94] Since xerostomia was defined by VAS > 80, this maybe a conservative estimate, leading to an underestimation of the condition.

Oral lichenoid lesions (OLL) were detected in 6 subjects (9%) with HCV infection. It is appropriate to consider the changes observed as OLL rather than OLP since the diagnosis of oral lichen planus is made only if clinical and histopathologic criteria are fulfilled. Prior studies have shown varying prevalence of OLP, ranging from 0% to 16%, in the HCV population.[93, 95-98] The prevalence of OLP in my study is unknown due to the lack of histopathological confirmation, but is no higher than 9% (the prevalence of OLL in my study). Incorporating a biopsy procedure in the study to rule out lesions other than OLP would have aided in eliminating false positive results and strengthen the results of the study.

(7) Conclusion

In conclusion, my study did not find an association between salivary hypofunction and chronic HCV infection. However, the feeling of oral dryness is high in HCV patients and this correlates with lower unstimulated salivary flow rates. Hence, there is a reason for health care providers to interview HCV infected patients regarding their subjective complaints of oral dryness as this may help identify those at high risk of developing dental complications and ensure that a more detailed evaluation by dentists will be performed. Furthermore, I found that severity of liver disease was associated with salivary gland dysfunction. There is a lack of understanding between salivary gland dysfunction and liver cirrhosis, except for a few studies done on alcoholic cirrhosis, which may not be representative of those with chronic liver disease due to infectious causes or metabolic causes. A better understanding of the mechanisms involved in salivary gland dysfunction in cirrhotic patients may help in minimizing oral complications of patients with chronic liver disease and allow better management of these patients.

(8) Tables and Figures

Table 1. Previous studies of the prevalence of salivary hypofunction and sialadenitis in HCV-infected patients [52, 53, 59,60]

Study done (Country)	Year	Study population; # of study subjects	Salivary flow measured (% with salivary hypofunction)	Histologic evidence of sialadenitis
Ferreiro et al (Spain)	2002	Internal Medicine clinic; N = 74	Whole Stimulated (16.6%)	Not done
Nagao et al (Japan)	2002	Internal Medicine clinic; N = 81	Whole Stimulated (23.4%)	Not done
Verdaan et al (Sweden)	1999	GI Liver clinic; N = 21	Whole Unstimulated (33%)	11%
Loustaud et al (France)	2001	GI Liver clinic; N = 45	Whole Unstimulated (62%) – using several different criteria for Sjogren's syndrome	47%

GI clinic: Gastrointestinal clinic

Table 2. Study population characteristics

	Cases HCV group (n = 76)	Controls Non-HCV group (n = 52)
Mean age (yrs) ± S.D	54.8 ± 8.2	52.4 ± 14.7
Gender (%) • Female • Male	 23 (30%) 53 (70%)	 21 (41%) 31 (59%)
Stage of Liver Disease (%) • No cirrhosis • Cirrhosis	 33 (44%) 43 (56%)	 22 (42%) 30 (58%)
Race (%) • Non-whites • Whites	 25 (33%) 51 (67%)	 44 (85%) 8 (15%)
Use of anticholinergic medication (%) • Yes • No	 25 (33%) 51 (67%)	 5 (10%) 47 (90%)
Use of any medication (%) • Yes • No	 70 (92%) 6 (8%)	 45 (87%) 7 (13%)

Table 3. Mean Unstimulated Whole Salivary Flow Rates in HCV and non-HCV Subjects

	HCV Group/Case (n=76) (ml/min ± S.D)	**Non-HCV group/ Control (n=52) (ml/min ± S.D)**
Mean UWSR	0.26 ± 0.15	0.30 ± 0.21
Stage of liver disease		
Non-cirrhotic	0.34 ± 0.18	0.27 ± 0.15
Cirrhotic	0.28 ± 0.17	0.22 ± 0.18
Gender		
Female	0.21 ± 0.11	0.25 ± 0.18
Male	0.29 ± 0.15	0.34 ± 0.21
Use of AC		
Not on AC	0.32 ± 0.18	0.27 ± 0.18
On AC	0.23 ± 0.15	0.19 ± 0.11

HCV: Hepatitis C infected
UWSR: Unstimulated whole salivary flow rate
AC: Anticholinergic medications
S.D: Standard deviation

Table 4. Comparison of Mean UWSR between Subject Subgroups

	n	Mean UWSR (mL/min)	SEM (mL/min)	P value*
Use of Anticholinergic Medication				0.01
Not on AC	98	0.30	0.02	
On AC	30	0.21	0.02	
HCV Status				0.23
Presence of HCV infection	76	0.26	0.02	
Absence of HCV infection	52	0.30	0.03	
Gender				0.01
Female	44	0.23	0.02	
Male	84	0.31	0.02	
Stage of Liver Disease				0.08
No cirrhosis	55	0.31	0.02	
Cirrhosis	73	0.26	0.02	

* using the unpaired Student's t-test
HCV: Hepatitis C virus
AC: Anti-cholinergic medications
S.E.M: Standard error of mean

Table 5. Variables Associated with UWSR in Univariate Linear Regression Analysis

Predictors	Coefficient, b	95% CI	P value	R^2
Male gender	0.08	0.02 - 0.14	0.01*	0.05
Use of anticholinergic medication	-0.09	-0.16 - 0.02	0.01*	0.05
Presence of cirrhosis	-0.54	-0.12 – 0.01	0.08	0.02
HCV status	-0.04	-0.1 – 0.02	0.22	0.01

*: $p<0.05$, statistically significant
CI : Confidence interval

Table 6. Variables Associated with UWSR in Multivariate Linear Regression Model

Predictors	Coefficient, b	95% CI	P value
Male gender	0.08	0.01 - 0.14	0.02*
Use of anticholinergic medication	-0.07	-0.15 - 0.00	0.04*
Presence of cirrhosis	-0.07	-0.12 – 0.00	0.03*
HCV status	-0.03	-0.09 – 0.03	0.35

$R^2 = 0.13$
*: $p<0.05$, statistically significant
CI : Confidence interval

Table 7. Comparison of mean VAS score for each item in the two groups

VAS Item (mm ± SEM)	Cases HCV group	Controls Non-HCV group	P - value
Oral Dryness	42.5 ± 3.1	25.4 ± 3.4	0.001
Oral Discomfort	35.7 ± 3.0	15.8 ± 2.8	0.001
Difficulty swallowing dry food	37.7 ± 3.4	22.0 ± 3.9	0.003
Difficulty swallowing any food	29.4 ± 2.9	9.1 ± 2.0	0.000
Difficulty speaking without drinking	27.1 ± 2.9	10.3 ± 2.7	0.000
Lip dryness	48.7 ± 3.2	33.7 ± 4.6	0.007

Table 8. Correlation Coefficients for UWSR and Individual VAS Scores in HCV Group

	UWSR	DRY	COMFY	DSDF	DSAF	DS	LD
UWSR	1.00						
DRY	-0.45*	1.00					
COMFY	-0.41*	0.72*	1.00				
DSDF	-0.41*	0.54*	0.56*	1.00			
DSAF	-0.35*	0.52*	0.55*	0.87*	1.00		
DS	-0.30*	0.46*	0.59*	0.64*	0.66*	1.00	
LD	-0.42*	0.64*	0.54*	0.51*	0.49*	0.42*	1.00

Legend:
UWSR: Unstimulated Whole Salivary Flow Rate
DRY: Oral dryness
COMFY: Oral discomfort
DSDF: Difficulty in Swallowing Dry Food
DSAF: Difficulty in Swallowing Any Food
DS: Difficulty in Speaking
LD: Lip Dryness
*: $p<0.05$ (Pearson's correlation)

Table 9. Correlation Matrix for the Correlation Coefficient between UWSR and Individual VAS Scores in Non-HCV Group

	UWSR	DRY	COMFY	DSDF	DSAF	DS	LD
UWSR	1.00						
DRY	0.00	1.00					
COMFY	-0.11	0.33	1.00				
DSDF	-0.20	0.30*	0.33*	1.00			
DSAF	-0.17	0.24	0.55*	0.58*	1.00		
DS	-0.28*	0.46*	0.48*	0.48*	0.58*	1.00	
LD	-0.12	0.52*	0.56*	0.40*	0.38*	0.36*	1.00

Legend:
UWSR: Unstimulated Whole Salivary Flow Rate
DRY: Oral dryness
COMFY: Oral discomfort
DSDF: Difficulty in Swallowing Dry Food
DSAF: Difficulty in Swallowing Any Food
DS: Difficulty in Speaking
LD: Lip Dryness
*: $p<0.05$ (Pearson's correlation)

Table 10. Oral Examination Findings

Locations	Oral Mucosal Changes	No. of subjects
Buccal Mucosa (bilateral)	• White, reticular striae	5 (5%)
	• Erosions/ Ulcers	1 (1%)
Attached gingiva	• White, reticular striae	1 (1%)

Figure 1. Correlation between UWSR (ml/min) and VAS score for oral dryness (mm) in non-HCV subjects

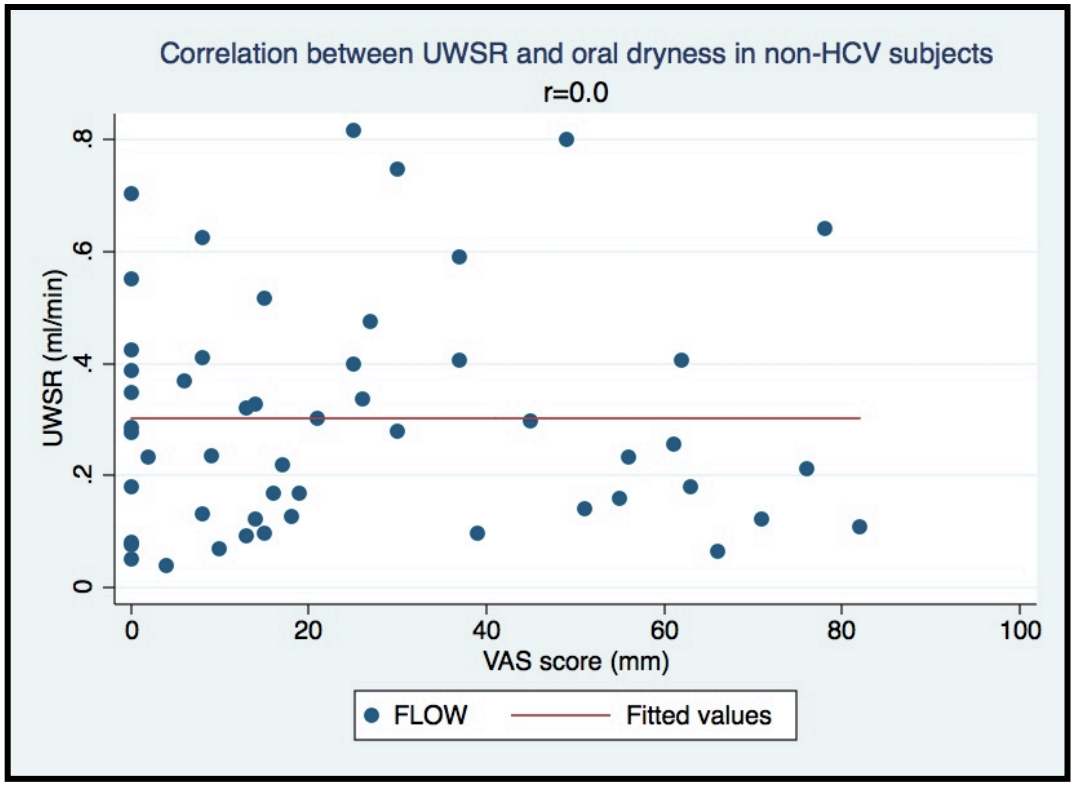


Figure 2. Correlation between UWSR (ml/min) and VAS score for oral dryness (mm) in HCV subjects

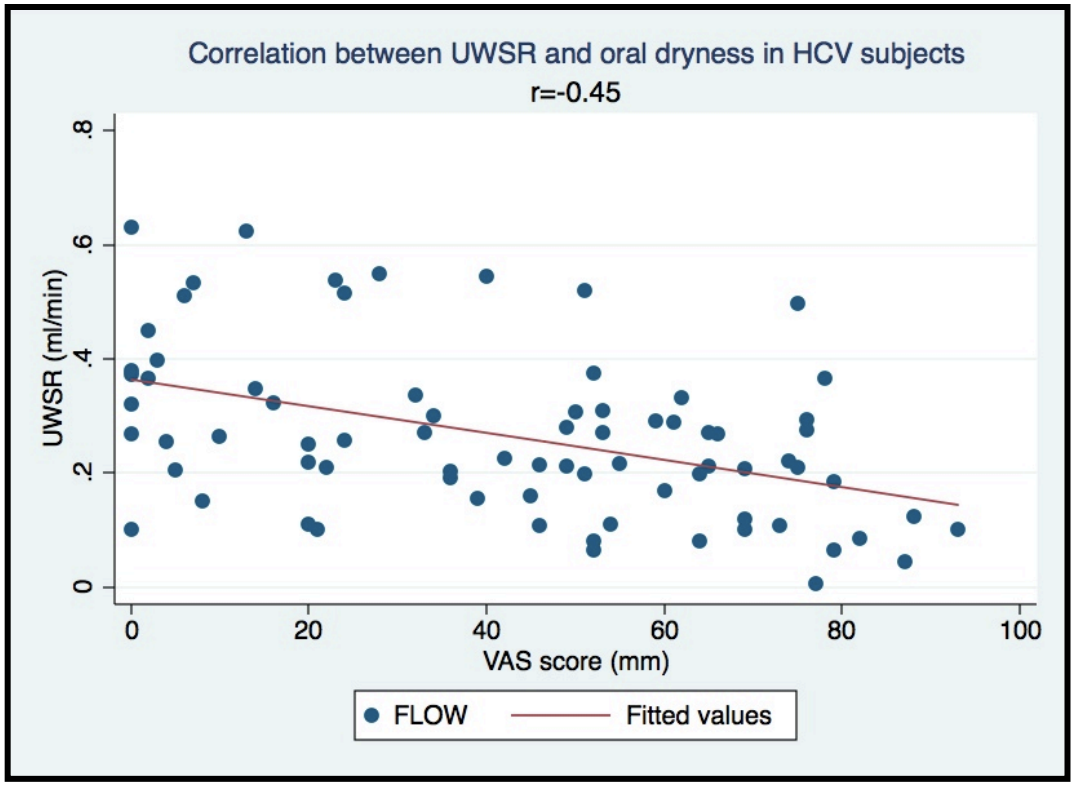


Figure 3. Correlation between UWSR (ml/min) and VAS score for oral discomfort (mm) in non-HCV subjects

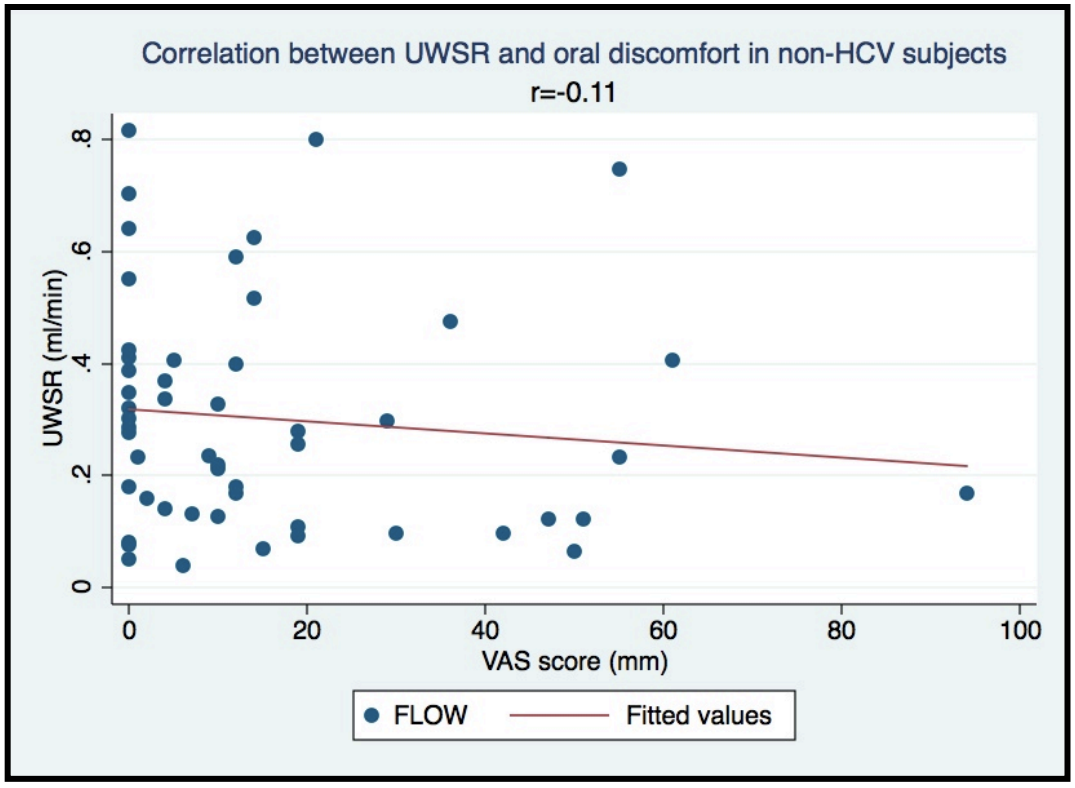


Figure 4. Correlation between UWSR (ml/min) and VAS score for oral discomfort (mm) in HCV subjects

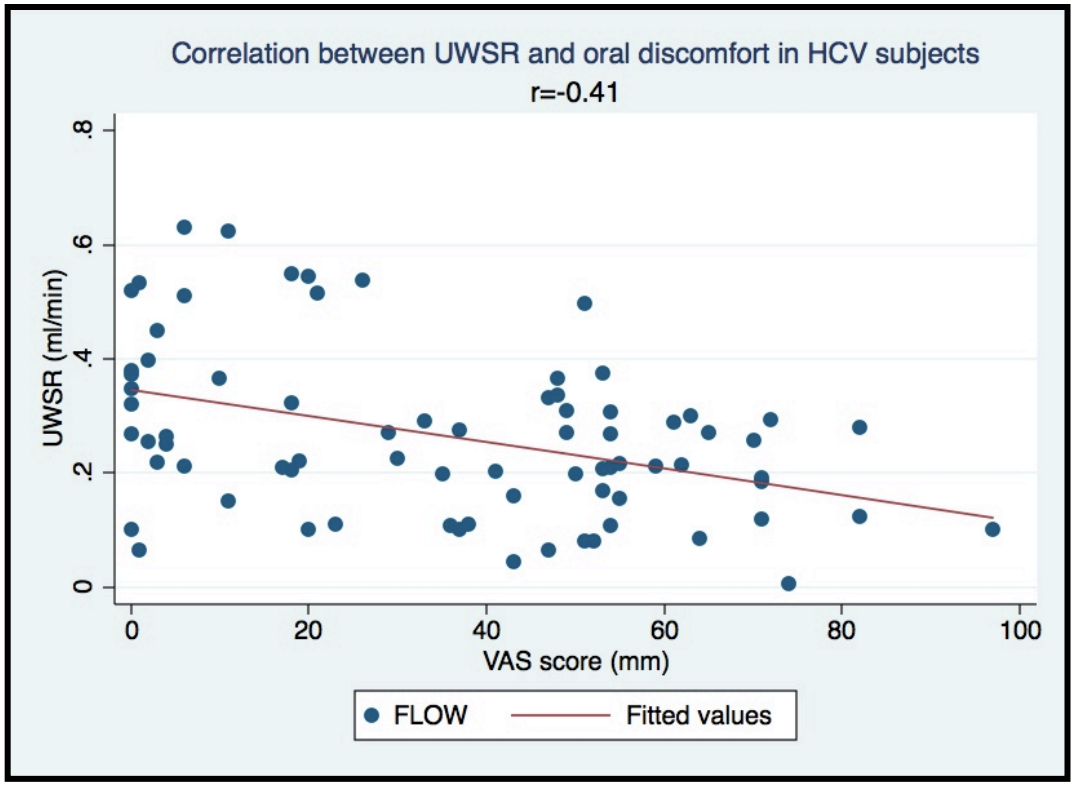


Figure 5. Correlation between UWSR (ml/min) and VAS score for difficulty in swallowing dry food without drinking (mm) in non-HCV subjects

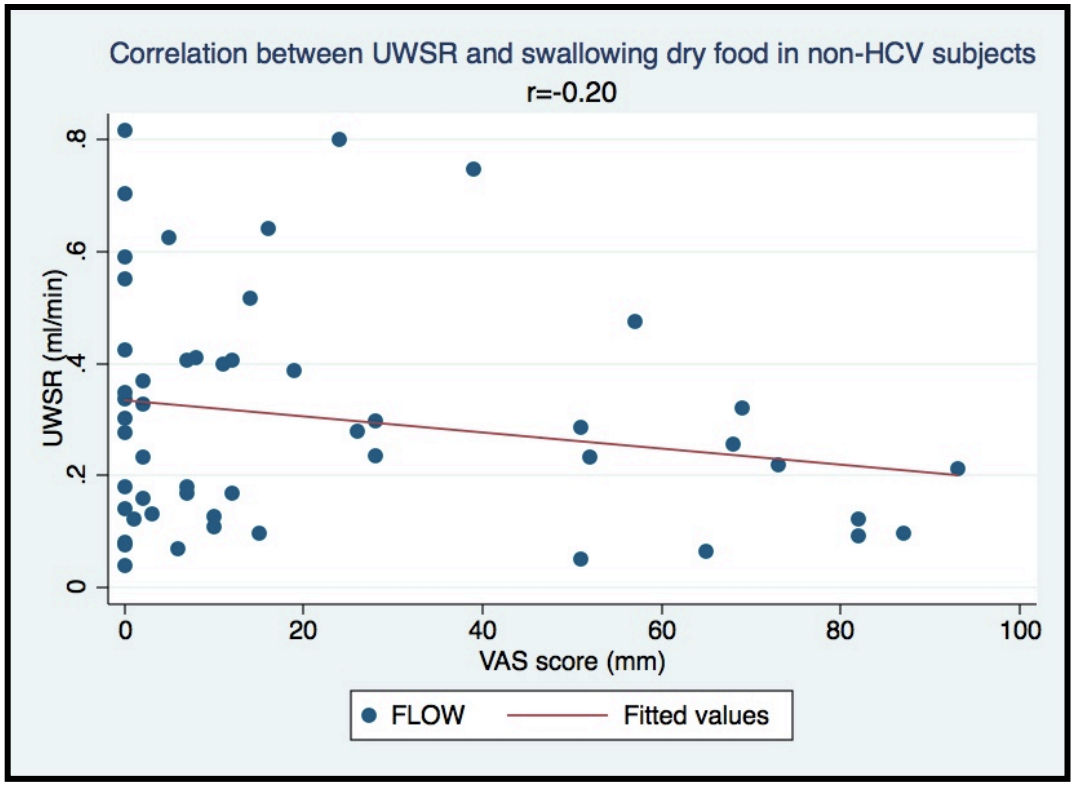


Figure 6. Correlation between UWSR (ml/min) and VAS score for difficulty swallowing dry food without drinking (mm) in HCV subjects

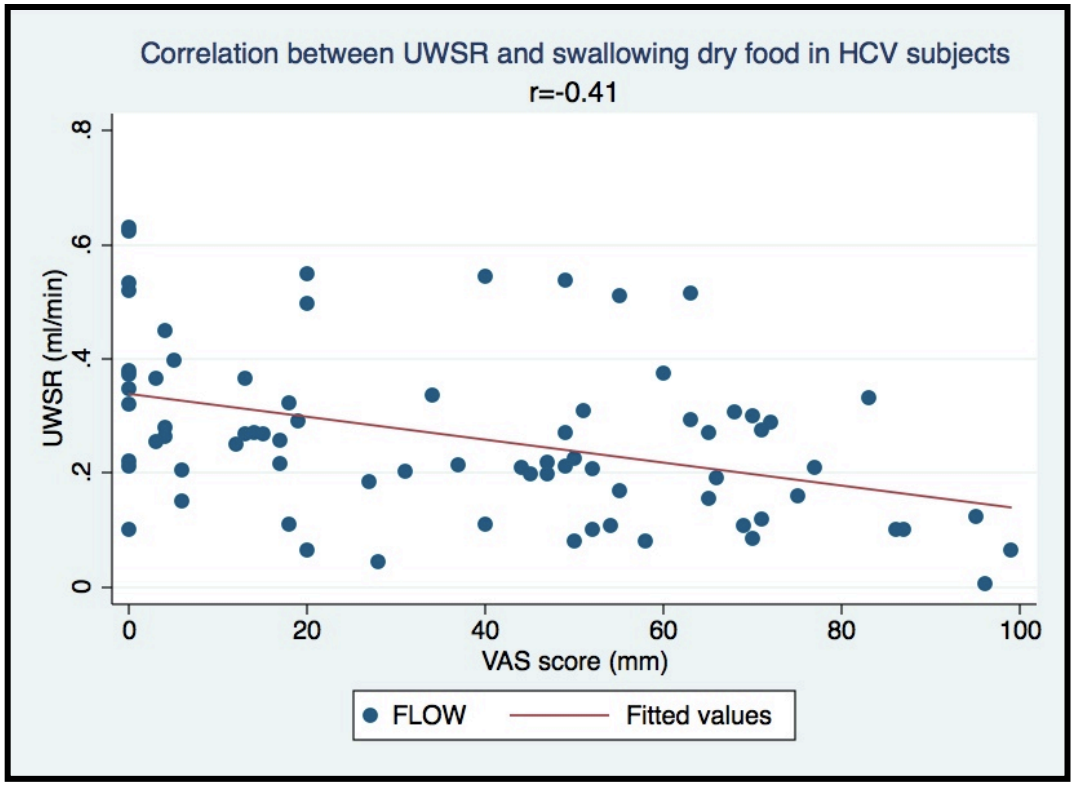


Figure 7. Correlation between UWSR (ml/min) and VAS score for difficulty in swallowing any food without drinking (mm) in non-HCV subjects

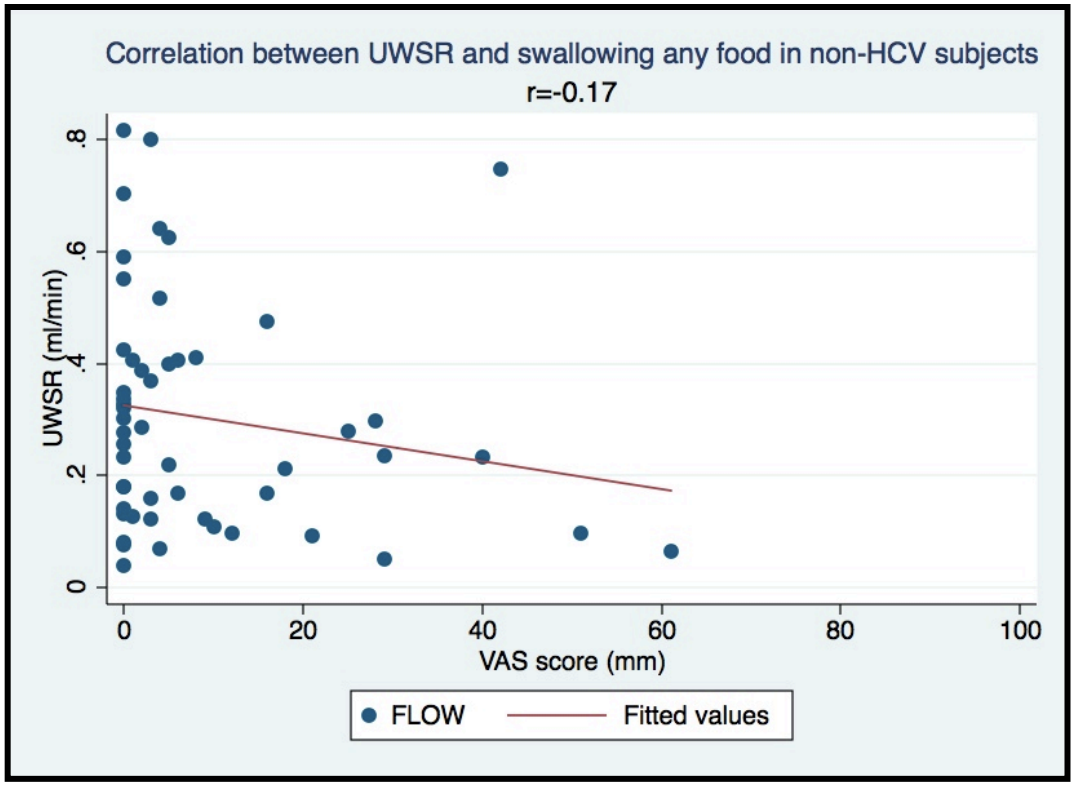


Figure 8. Correlation between UWSR (ml/min) and VAS score for difficulty in swallowing any food without drinking (mm) in HCV subjects

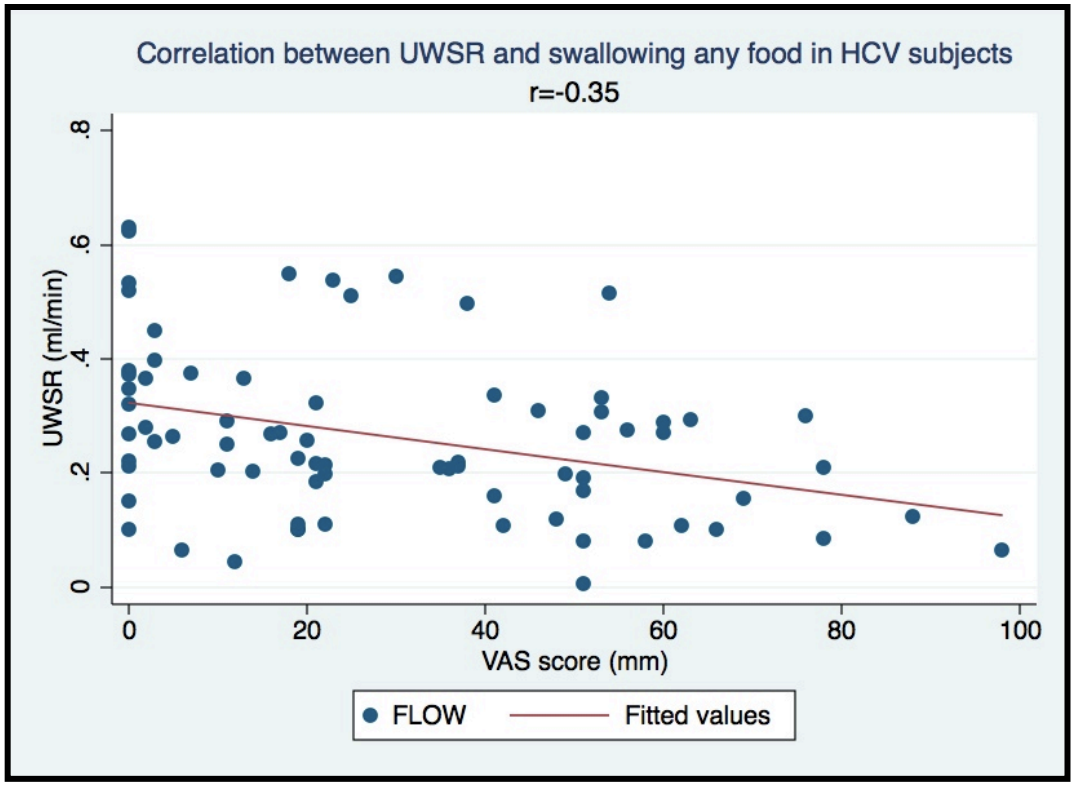


Figure 9. Correlation between UWSR (ml/min) and VAS score for difficulty in speaking without drinking (mm) in non-HCV subjects

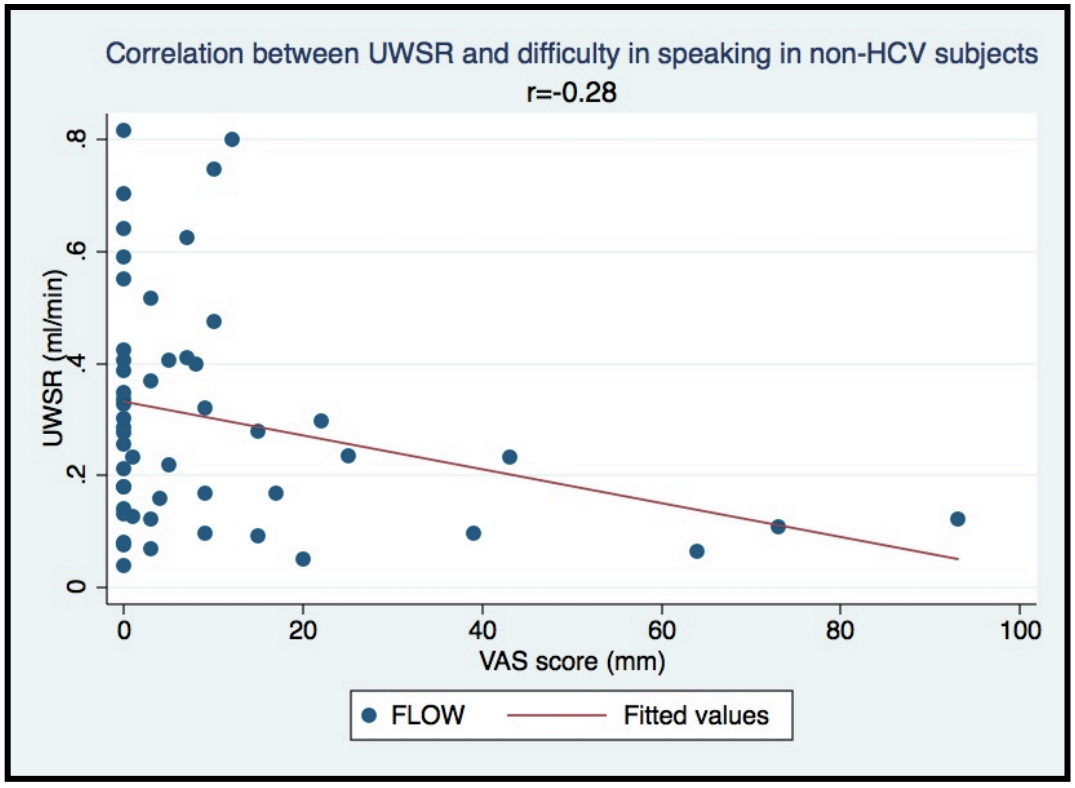


Figure 10. Correlation between UWSR (ml/min) and VAS score for difficulty in speaking without drinking (mm) in HCV subjects

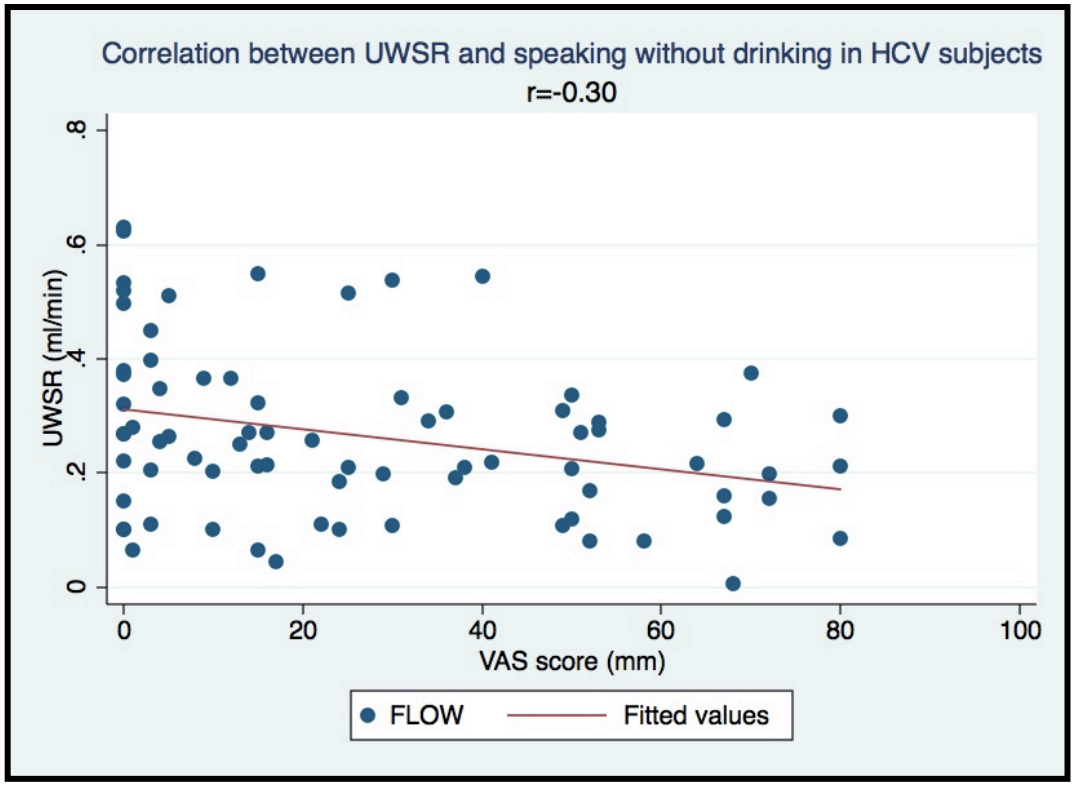


Figure 11. Correlation between UWSR (ml/min) and VAS score for lip dryness (mm) in non-HCV subjects

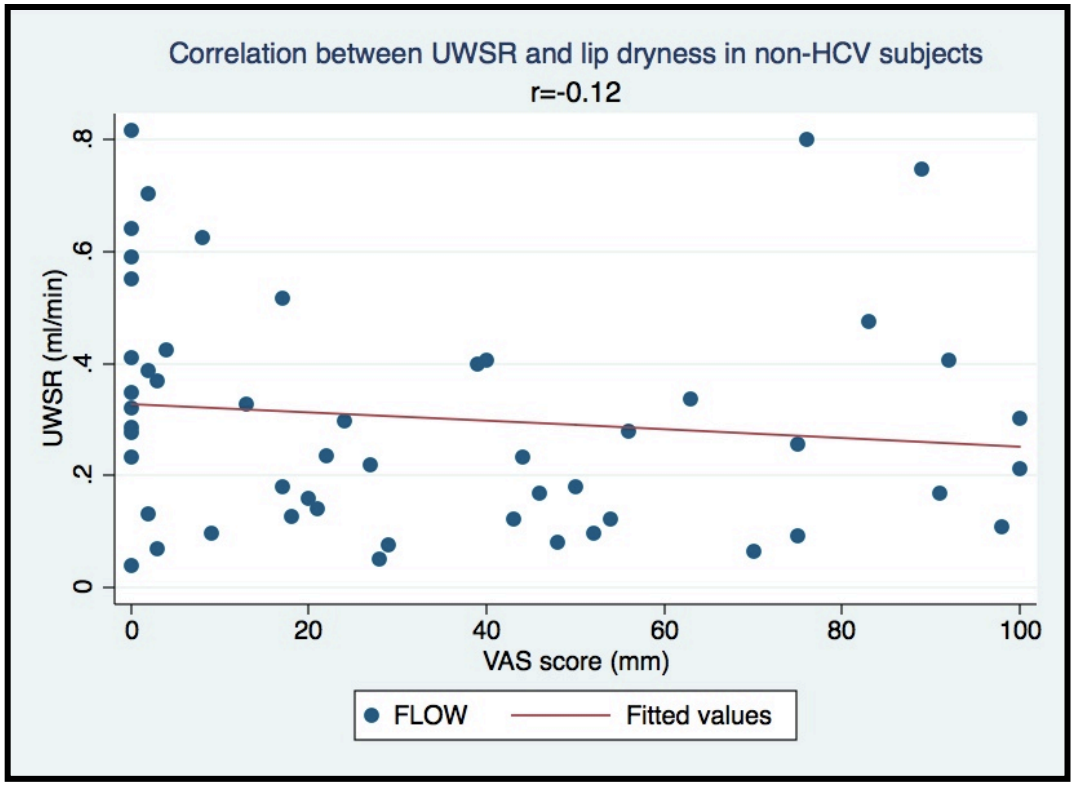
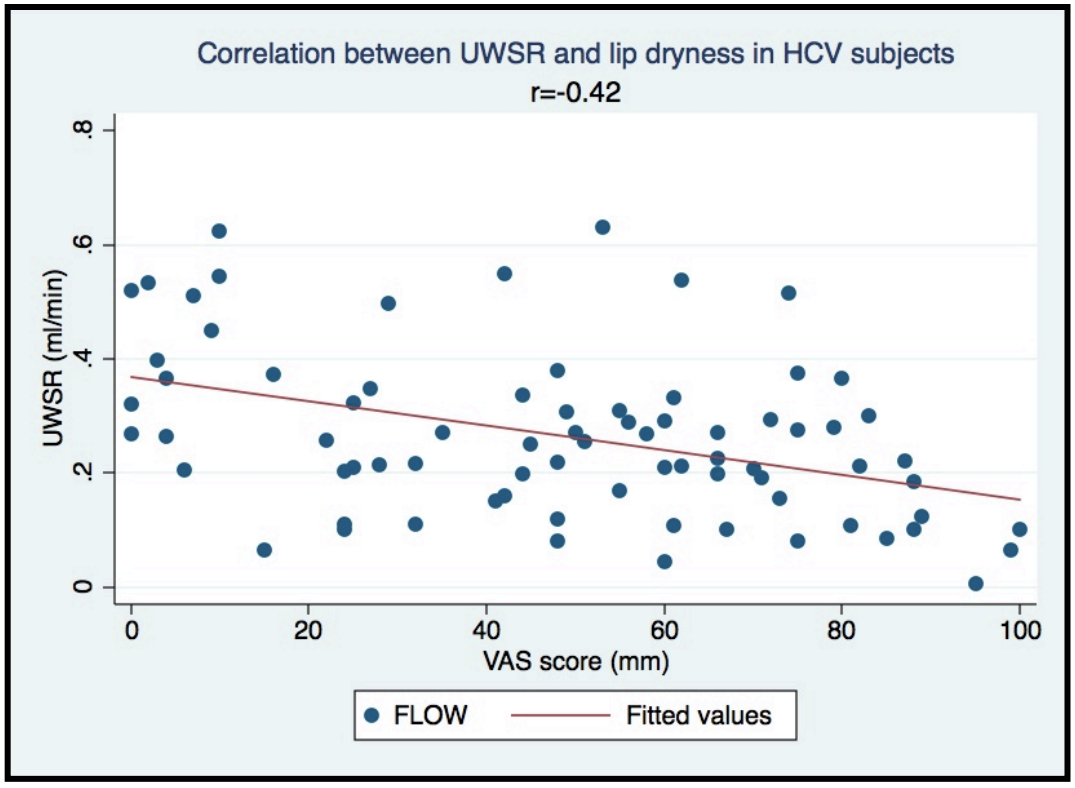


Figure 12. Correlation between UWSR (ml/min) and VAS score for lip dryness (mm) in HCV subjects, r = -0.42



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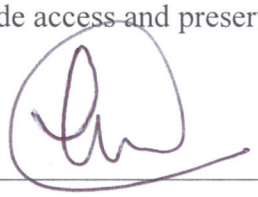

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