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Research Article

Salivary PH Changes in Liver Cirrhosis and Hepatocellular Carcinoma

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Abstract

Reports in recent years have indicated that saliva represents an increasingly valuable resource for disease diagnostics, including periodontal diseases, as well as different types of cancer, cardiovascular, endocrine, immune, and hereditary diseases. In contrast to blood pH which is under tight control, salivary pH shows variability depending on a wide range of factors reflecting its potential variability in chronic health status.

Aim of study: To investigate salivary pH changes in patients with liver cirrhosis and hepatocellular carcinoma (HCC).

Methods: Salivary pH was measured in 300 subjects using narrow range pH strips. Subjects were divided into 3 groups, group I included 100 healthy volunteers, group II included 100 patients with liver cirrhosis and group III included 100 patients with HCC.

Results: A significant difference ($P \leq 0.05$) in salivary pH values was found between the three groups. The HCC group had the most acidic value (5.62 ± 0.26), followed by the cirrhotic group (6.24 ± 0.40). The control group showed normal salivary pH (6.6 ± 0.53). The salivary pH level at a cut off value ≤ 5.85 gives sensitivity = 80% and specificity = 84% to predict presence of hepatic malignancy among patients with liver cirrhosis.

Conclusion: A state of chronic tissue acidosis is present in patients with liver cirrhosis which become more profound with development of HCC.

Keywords: *Salivary Ph; Liver Cirrhosis; Hepatocellular Carcinoma*

Introduction

PH value refers to the concentration of [H^+] ions in a solution. The pH of the blood is perhaps the most tightly regulated process in human physiology. In contrast, salivary pH shows more variability depending on a wide range of factors including diet, stress, medications and diseases. Other tissues also show variability in their pH depending on the mentioned factors [1]. Many authors consider the salivary pH to be a mirror of tissue pH and several studies were carried out to explore this notion [2-5].

Some investigators studied salivary pH in relation to systemic diseases that are associated with acidic element which is assumed to reflect on the oral cavity such as in gastroesophageal reflux disease, bulimia nervosa and burning mouth syndrome [6]. Others studied chronic renal failure patients on peritoneal dialysis with and without diabetes mellitus [7]. In patients with bronchial asthma, significant changes in salivary pH were found compared to controls [8].

A number of studies using pH-sensitive magnetic resonance imaging contrast agents, microelectrodes, and magnetic resonance spectroscopy with hyperpolarized C-13 have consistently shown that the extracellular pH (pHe) of tumors is significantly lower (6.6 - 7.0) than

that of healthy tissues (7.2 - 7.4) [9-12]. Thus, salivary pH changes in malignancy were also an attractive subject to explore.

The tissue acidity associated with malignancy was explained primarily by (a) anaerobic glycolysis in tumor regions subjected to short-term or long-term hypoxia as a result of poorly organized vasculature with diminished blood flow, and (b) aerobic glycolysis (the Warburg effect), which is a common cancer phenotypic property in which the glycolytic metabolic pathways are used even in the presence of oxygen [13-14].

Interestingly, tumor cells are typically able to maintain high proliferation rates even in an acidic environment [15]. An acidic pHe, on the other hand, induces significant toxicity in normal cells by reducing proliferation and promoting apoptosis via a p53- dependent pathway initiated by increasing caspase activity [16].

It is becoming increasingly evident that acidosis plays an important role in the somatic evolution and progression of cancer from pre-invasive to malignant disease. Early studies by Morita et al. described the clastogenic properties of low pHe on mammalian cell lines in vitro, a clastogenic is a mutagenic agent causing disruption of chromosomes, these studies concluded that not only is the extracellular pH for tumors affects release of enzymes, proteolytic agents and alters normal cellular metabolism but also causes chromosomes mutation rendering the cells more carcinogenic [17].

In addition, some data confirms the alteration of salivary pH in individuals on a more acidic (high protein) versus more alkaline (more vegetables diet) [18]. A trend of alkalizing one's body through an alkaline diet or alkaline water to achieve better health and control chronic diseases is currently developing.

Aim of the Study

To investigate salivary pH changes in patients with liver cirrhosis and hepatocellular carcinoma.

Subjects and Methods

This is a cross sectional study designed to compare salivary pH in patients with post hepatitis liver cirrhosis, patients with hepatocellular carcinoma (HCC) with normal subjects. The study included 300 subjects who were recruited from Hepatology Department of Ain Shams University Hospitals, during the period from August 2013 to January 2015. Included subjects were arranged in three equal groups (100 subjects in each group):

Group (I): healthy volunteers (Control group).

Group (II): patients with liver Cirrhosis (post viral hepatitis)

Group (III): hepatocellular carcinoma.

For all patients; Child Pugh score and MELD score were calculated to evaluate severity of liver disease [19].

Patients with HCC were staged according to Barcelona clinic liver cancer (BCLC) staging system [20].

All enrolled subjects were asked to sign an informed consent before participation in the study.

The study protocol was approved by the ethical committee of Ain Shams University hospitals.

Exclusion Criteria

All patients with chronic diseases that are known to alter pH level; Diabetes mellitus, renal failure, gastroesophageal reflux disease, bulimia nervosa, respiratory failure, decompensated heart failure, smokers.

Principles of pH measurement

Salivary pH measurement was performed by using pH-indicator strips color pHast of the narrow range type pH 4.0 - 7.0 provided by EMD chemicals, Germany. These strips give sharp and clear color changes and are matched to a color scale provided on the box; each color scale corresponds to the following specific pH numerical values 4.0, 4.4, 4.7, 5.0, 5.3, 5.5, 5.8, 6.1, 6.5, 7.0.

Collection of saliva specimen

Unstimulated saliva is collected within the subject's buccal cavity. Asking the subject to collect and swallow the saliva within the buccal cavity twice before testing the third collection. A pH strip is placed into the saliva collection inside the mouth for 30 seconds after which it is taken out, wiped off and compared to the color scale to determine pH value.

Test was avoided from 0-2 hours' post meal.

Expected Normal Value

The pH value for the salivary fluid of a healthy individual is known to be in the range of 6.5 to 7.5. Optimum values are those closer to 7.5 (alkaline range).

Statistical Methods

- Data were analyzed using PASW statistics software ver. 18.
- Quantitative data were expressed as Mean ± S.D.
- Qualitative data were expressed as number of cases and their percent of expression.
- Comparative analyses were done using (Student t, ANOVA) tests and (Mann whitney, Kruskal Wallis) tests for parametric and non-parametric
- When significant difference was found between the three groups, post Hoc test was used to define the statistical difference between subgroups using APA-style notation; If a pair of values is significantly different, the values have different subscript letters assigned to them. If the three groups show statistical difference in pair comparisons, different letter (A, B, C) was assigned to each.
- Correlations were done using Pearson's correlation coefficient.
- Multivariate linear regression analysis was done to determine the independent variables which significantly correlated with patients' salivary PH.
- ROC curve analysis was done to estimate the predictive performance of salivary PH as marker of hepatic malignancy.

Results

Table 1 shows age and sex distribution among included patients. Child Pugh class and mean MELD score of patients in group II and III is also presented in the same table.

Variables		Group I (normal)	Group II (cirrhosis)	Group III (HCC)
Age (years) (mean ± SD)		45.48 ± 8.26	50.4 ± 7.7	56.99 ± 6.34
Sex	Males	70	74	74
	Females	30	26	26
Child Pugh (n&%)	A	NA	39(%)	19(%)
	B	NA	48(%)	47(%)
	C	NA	30(%)	23(%)
MELD score (mean ± SD)		NA	14.75 ± 4.32	11.78 ± 4.05

Table 1: *Descriptive data of the studied patients*

SD: standard deviation NA: not applicable

Table 2 shows comparison of the salivary pH among the three groups; a significant difference in mean salivary pH value found between all groups, with the HCC group being significantly more acidic than the other two groups.

Variables	Group I (normal)	Group II (cirrhosis)	Group III (HCC)	P value
Salivary pH (mean ± SD)	6.6 ± 0.53^{A}	6.24 ± 0.40^{B}	5.62 ± 0.26^{C}	≤ 0.01

Table 2: *Descriptive data of the studied patients.*

ABC = the three groups show statistical difference in pair comparisons, hence, the different letters assignment.

Figure 1 shows a significantly lower salivary pH in Child A patients of HCC group (5.62 ± 0.26) compared to corresponding Child A in group of liver cirrhosis (6.4 ± 0.4). Similarly, Child B patients in HCC group have significantly lower salivary pH (5.7 ± 0.26) compared to Child B patients in the cirrhotic group (6.2 ± 0.33). Child C patients show that same significantly lower salivary pH (5.52 ± 0.24) in HCC group compared to cirrhotic group (5.75 ± 0.18).

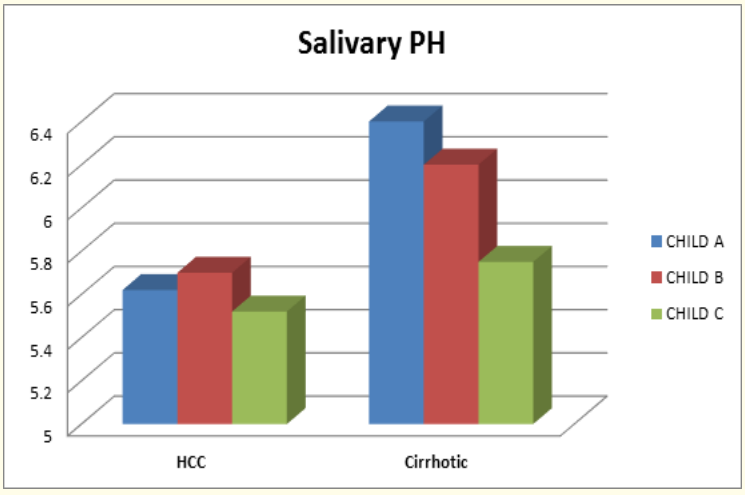

Figure 1: *Salivary pH in different Child Pugh Class in group of liver cirrhosis and HCC group (P ≤ 0.01).*

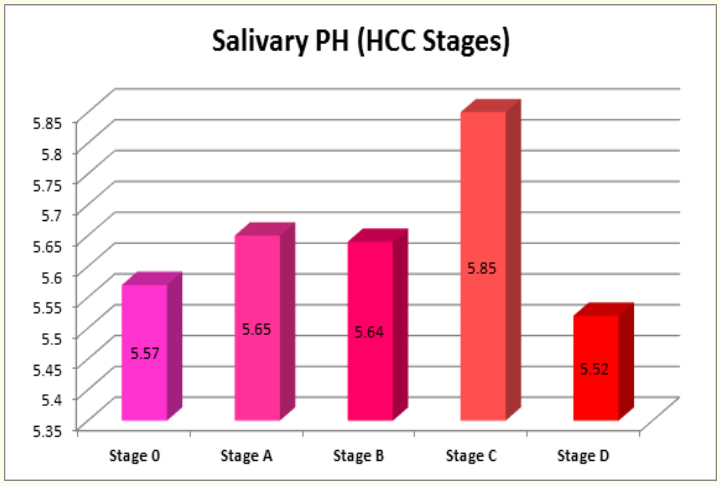

Figure 2: *Salivary pH in different stages of HCC according to BCLC staging (P > 0.05).*

Figure 2 shows no significant difference in HCC patients' salivary pH in relation to the BCLC stages.

In table 3 multivariate linear regression analysis was done to determine the independent variables which significantly correlated with patients' salivary pH. It showed that age, total bilirubin, and MELD score were independent predictors to patients' salivary pH.

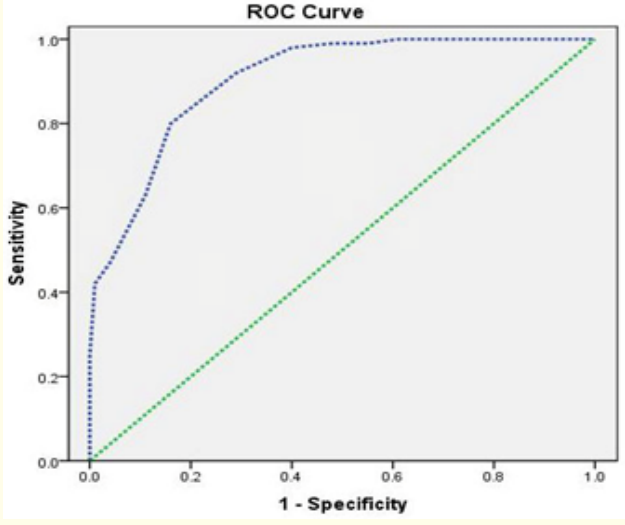

Figure 3: *Roc Curve for salivary pH for diagnosis of HCC.*

ROC curve analysis was done to estimate the predictive performance of salivary PH as marker of hepatic malignancy among patients with liver cirrhosis (group II &III). It shows that the pH level at a cut off value of ≤ 5.85 gives a sensitivity of 80% and a specificity of 84%.

Cut off	AUC 95% CI	Sensitivity	Specificity	PPV	NPV
≤ 5.85	90.5% (86.6 – 94.5)	80%	84%	83.3%	80.8

Table 4: *Analysis of Roc Curve of salivary pH for diagnosis of HCC.*

PPV positive predictive value NPV negative predictive value

Discussion

The Salivary fluid has become very popular recently in the field of diagnostics, with many discoveries on the salivary composition and its diagnostic use in many systemic diseases [21]. Most studies in this regards used a marker for a specific disease in salivary fluid in co-relation with its serum marker to determine its diagnostic value such as salivary alpha feto protein, salivary CA15.3 and c-erbB-2 in patients with breast cancer [22-23].

Salivary pH however, poses a more challenging context as it doesn't reflect blood pH directly because blood pH is under very tight control when compared to other biochemical changes that occur in the blood. Therefore we cannot use blood pH as an indicator for chronic diseases nor as a reflection of tissue pH.

The current study aimed to investigate whether there is any change in salivary pH in patients with liver cirrhosis in general and liver malignancy in specific and the possible use of salivary pH as an indicator of liver disease severity and presence of HCC.

Analysis of our data showed that enrolled subjects were gender matched but had difference in their mean age; the relatively big number of subjects included (300 subjects) together with the sequential enrollment of them, made it difficult to ensure accurate matching. In addition, HCC may need longer time to develop after established cirrhosis, as a result, HCC patients had older age. We supposed that bias in results due to age difference is minimal.

The salivary pH in the three groups showed a significant difference among them in comparison to each other. The HCC group showed a highly significant lower salivary pH compared to the group of liver cirrhosis and control group. HCC group had the most acidic pH value. The cirrhotic group showed more acidic values when compared to control and less acidic values when compared to the HCC group. The healthy control group came out to be the least acidic than the two diseased groups and its mean range was found out to be in the normal values of salivary pH (6.5 ± 0.53) [24]. Our results supported the postulation that salivary pH shows significant acidic changes in malignancy and chronic diseases.

We further compared salivary pH in patients' group II and III after re-classifying them according to disease severity using Child Pugh scoring system. We found that the salivary PH was significantly more acidic in all HCC patients in different Child Pugh stage compared to their corresponding that had cirrhosis without HCC. However, salivary pH in group II and III progressively became more acidic with advancement in stage of liver cirrhosis. The above observations suggest that salivary pH is significantly more acidic in cases of malignancy than cases of chronic liver disease despite a common clinical stage and almost equal status of liver function. On the other hand, study of salivary pH in different BCLC stages of HCC patients did not show statistically significant difference. This may indicate that malignancy causes a significant drop in tissue pH regardless its clinical stage.

According to table 3, age was one of the independent variables to salivary pH. It shows a significant negative correlation to salivary pH. A possible explanation for this could be that salivary pH reflects the aging process on a cellular and metabolic level. It is known that changes in the metabolic capacity of cells and tissues occur with aging, examples include changes in the salivary composition and sig-

nificant decrease in salivary antioxidant capacity in elderly individuals [25]. Changes in the salivary flow rate and buffering capacity of unstimulated saliva significantly drop with aging [26] thus, contributing to rise in acidity of salivary fluid with age.

Serum bilirubin and INR are parameters used in grading disease severity in Child score and MELD model. In cases of cirrhosis and HCC, their rise is most often representing to the beginning of failure of hepatic units. The inverse relation between serum bilirubin, INR and MELD value with the salivary pH explains our results which showed increase in acidity and drop in salivary pH with progression of liver disease.

Serum creatinine level is directly related to kidney function; it makes sense to consider how any mild impairment in kidney functions will impact the acidity of body tissues and fluids, since the kidneys are the prime strongest system in acid-base balance. Moreover, creatinine rises with the progression of liver disease. Thus, it's logical to find serum creatinine as one of determinants of salivary pH with significant inverse correlation between both values (serum creatinine and salivary pH).

MELD is a scoring system to measure mortality risk in patients with end stage liver disease, and it involves more than one indicator, creatinine, bilirubin and INR. Since all its parameters affect the salivary pH, MELD value has a strong negative correlation with salivary pH.

In our study, we found that the predictive performance of salivary pH as a marker for hepatic malignancy among all patients (group II & III) has a sensitivity of 80% and a specificity of 84% at a cut off value of ≤ 5.85.

In summary, salivary pH was in the acidic range in patients with liver cirrhosis and significantly more acidic in patients with HCC. Salivary pH decreases progressively with advance in liver disease severity as indicated by Child Pugh classification. The non-invasive and economically sound nature of salivary pH makes it a potential auxiliary test in screening for HCC. Finally, alkalinizing body tissues in patients with chronic liver disease may be a possible therapeutic target in the coming future.

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