Validation of a Salt Sensitivity Test as a Possible Alternative to a 24-Hour Urine Test

Rada Mazaheri1*, Morteza Abdollahi2, Arezoo Rezazadeh1, Hassan Eini-Zinab1*

Abstract
Objectives: The 24-hour urine sodium (Na) test is the gold standard method for measuring salt intake in community studies. This study aimed to compare the results of a 24-hour urine Na test with those obtained from the salt taste sensitivity test (STST) in order to validate the STST as a possible substitute for the 24-hour urine Na test.

Materials and Methods: Both tests were simultaneously performed on 30 individuals. The results were compared using correlation models.

Results: STST results in the tolerance threshold had a strong and positive correlation with the results of the 24-hour urine Na (r=0.93, P<0.001). In addition, the distance between the tolerance threshold and the recognition threshold scores was strongly correlated with the results of 24-hour urine Na (r=0.830, P<0.001). Finally, there was no significant correlation between recognition threshold scores and 24-hour urine Na (r=0.087, P=0.64).

Conclusions: In general, the STST showed a promising potential to be replaced with the 24-hour urine Na test in community studies.

Keywords: Dietary sodium intake, Salt taste sensitivity test, Salt tolerance, Taste threshold, Urine sodium, validity

Introduction
Sodium (Na) can be obtained from different sources. Some foods naturally contain this element. Moreover, Na is added to foods during processing, preparation, and cooking steps in the form of Na chloride. Numerous studies have identified the correlation between salt intake and high blood pressure in patients suffering from renal failure, liver disease, and congestive heart failure (1-3). The interaction between genetic predisposing factors and bad eating habits plays a key role in the onset and progression of the aforementioned diseases. In this light, high Na intake has been suggested as an independent risk factor for cardiovascular diseases and gastric cancer. Therefore, limiting the dietary intake of Na can be an effective way to prevent and control hypertension and reduce the incidence of gastric cancer (3-8). Increased dietary salt intake in animal models also results in increased oxidative stress. Taking together, increasing salt intake in the daily diet has become a global concern (9).

Several methods are used to measure the salt intake in community studies, including food recalls, the food frequency questionnaire, and a 24-hour urine test (10). Among them, the 24-hour urine collection test is the most reliable method in the context (10,11). This method has been used in a wide variety of studies such as physiological, metabolic, and epidemiological investigations. This method can accurately reflect the Na content absorbed from different sources. However, the use of a 24-hour urine collection test in large-scale studies is problematic which requires to be replaced with simpler approaches. Furthermore, this method only indicates the current consumption of Na (11).

Some studies evaluated salt intake based on the subjective measuring of salt content in the consumed foods (7,12,13). The ability to sense the taste of salt can affect the tendency to salt consumption. The main problem in assessing Na intake through the questionnaire is that people usually ignore such history or fail to recall their diet in the past. Salt flavor preferences are personal characteristics and may eventually be a practical way for evaluating salt intake. Salt taste sensitivity test (STST) is used for the prediction of high blood pressure. It is shown that people with high blood pressure are significantly less sensitive to the taste of salt compared to those with normal blood pressure. There is also a correlation between high sensitivity to the salt taste and the over-consumption of Na (13). The taste recognition threshold is a concentration in which a person recognizes the taste of a flavor. The desire to eat Na is a strong motivation for consuming salt. Taste can be the main determinant of food choice. The Intersalt study suggested that lower Na intake may have positive effects on lowering...
blood pressure, age-related blood pressure, and eventually, mortality from cardiovascular disease. It is also reported that patients with exercise-induced hypertension have a higher STST result (14). Given the importance of the salt intake measurement and the limitations of the 24-hour urine test, there is an urgent need for developing a simple, fast, reliable, and less expensive method regarding determining salt intake, especially for community-based studies. Accordingly, this study sought to assess the validity of STST as an alternative method for the 24-hour urine test.

**Materials and Methods**

**Participants**
In this cross-sectional study, 30 candidates including 15 women and 15 men aged 24-68 years, working in different work offices of Zabol with a diploma to a master’s degree were selected based on study objectives. None of the participants had chronic disorders and or consumed tobacco and drugs. Written informed consent was taken from all study subjects. People were excluded from the study if they had a history of hypertension while not taking any medications, uncontrolled hypertension despite treatment, acute oral problems, and started taking new medications in the last month, along with the women who were menstruating.

**Method of Performing a 24-Hour Urine Test**
Plastic containers for the 24-hour urine test containing preservatives were provided to the participants. All necessary information regarding the steps of urine collection was provided to volunteers and they were also recommended to record the beginning and ending time of the urine collection in the donor’s datasheet.

At this stage, 15 people were excluded from the study because of the improper collection of 24-hour urine or failure to deliver the sample at the mentioned time. They were replaced with similar subjects in the terms of age and gender. To avoid interference with 24-hour urine test results, all 24-hour urine tests were performed before the salt sensitivity test. The 24-hour urine Na was applied as a gold standard test for assessing the validity of the salt test. Urine creatinine was also measured to confirm the proper collection of urine (15). The volume of the collected urine during 24 hours (mm per 24 hours) and the amount of Na (mEq per 24 hours) were calculated as well.

**Preparation of Salt Solution**
Double distilled water and Na chloride were used to prepare salt solutions at the concentrations of 0.22, 0.45, 0.9, 1.8, 3.6, 7.3, 14.6, 29.2, and 58.4 g/L. Then, the solutions were stored in glass containers at 5°C. All solutions were weekly prepared and placed at room temperature before consumption. The solutions were numbered from 1 to 9 based on low to high concentrations, respectively. The tap water was considered as the control solution (number 0) (Table 1).

**Salt Sensitivity Test**
Nilsson’s method was used to test the sensitivity to the salt flavor (13,16), and the salt sensitivity test was conducted in the morning by a trained nutritionist. At the beginning of the test, all stages were explained to the volunteers. To avoid any bias, participants were not informed about the taste of solutions. They were recommended to wash their mouths with tap water. The control solution was tasted at the beginning of the test, followed by the other solutions, ascendingly. For this purpose, five drops of the salt solution were poured into the mouth with a dropper, and after 30 seconds, the person was asked about the flavor. If the answer was yes, the subject had to choose the taste of the solution as sour, salty, bitter, water (no flavor).

After answering these questions, the person could wash his/her mouth with water, and these steps were repeated for all solutions. The first solution was considered as the first person’s score on the test if that person could accurately determine its level of salinity (the recognition threshold). Later, the test was continued until the person defined the taste of a solution as extremely salty (the threshold of tolerance). If a person did not identify any of the solutions as highly salty, solution 9 was selected as the second threshold (tolerance). One hour before the test, people were asked to not consume anything other than water, including tobacco, toothbrushes, and gums. All subjects were tested for 4 hours from 8 am to 12 noon.

**Behavioral Questions**
To determine the distribution of the study subjects regarding their preferential taste, they were questioned about the use of salt in the food table.

**Clinical Parameters**
Blood pressure was measured by a mercury sphygmomanometer in two stages, namely, before the salt sensitivity test in a sitting position to ensure that the person’s blood pressure was in the normal range and 30 minutes after the end of the test to measure changes in blood pressure after the salt sensitivity test. Blood pressure was measured twice, and the average for each stage was calculated in each stage. Hypertension in adults was defined as mean systolic blood pressure ≥140 mm Hg, mean diastolic blood pressure ≥90 mm Hg, or current treatment with antihypertensive medications either at the time of the interview or in the previous month. Anthropometrical measurements were taken barefoot.
while wearing light clothing. Their weights and heights were measured according to the standard protocol. The body mass index was calculated by dividing the weight (kg) by the square of height in meters (17).

**Statistical Method**

Statistical analysis was conducted using STATA-14 and SPSS (IBM SPSS Statistics for Windows, version 21.0). Given the non-normally distribution of quantitative variables, the Spearman correlation test was applied to estimate the correlation between STST scores and urine Na. Moreover, the comparison of mean urine Na between different scores of recognition and the tolerance threshold was performed using the Kruskal-Wallis test. Then, the coefficients of the correlation between genders were compared by applying interaction terms in linear regression models. The $P$ value less than 0.05 was considered statistically significant.

**Results**

In general, 30 people were studied, 50% of whom were males. On average, men were significantly taller than women in the study (173.42 cm vs. 162.61 cm, respectively, $P=0.001$). Men also showed higher amounts of Na in their urine samples compared with female participants (137.06 vs. 109, respectively, $P=0.039$). Other clinical and demographic characteristics were the same among men and women (Table 2).

There was a significant difference between the recognition score of females and males ($P=0.047$) while no significant difference was observed in the tolerance threshold ($P=0.061$).

As shown in Table 1, the mean urine Na was higher in patients with scores 3 and 4 of the recognition threshold, but the difference was not statistically significant ($P=0.313$). However, the urine Na significantly increased with the increase in the tolerance threshold scores ($P<0.001$) and the distance between recognition and tolerance thresholds ($P=0.001$).

There was a non-significant correlation between recognition threshold scores and 24-hour urine Na in all subjects ($r=0.087$, $P=0.64$), men ($r=0.458$, $P=0.086$), and women ($r=0.247$, $P=0.374$). However, the tolerance threshold score had a strong and significant correlation

<table>
<thead>
<tr>
<th>STST Steps</th>
<th>Recognition Threshold</th>
<th>Tolerance Threshold</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>P Value</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>0</td>
<td>85 (29.60)</td>
</tr>
<tr>
<td>2</td>
<td>74.5 (33.23)</td>
<td>0</td>
<td>91 (28.59)</td>
</tr>
<tr>
<td>3</td>
<td>127.69 (38.94)</td>
<td>0</td>
<td>110.8 (24.69)</td>
</tr>
<tr>
<td>4</td>
<td>125.83 (38.92)</td>
<td>0.313</td>
<td>120 (2.83)</td>
</tr>
<tr>
<td>5</td>
<td>124 (12.29)</td>
<td>73.14 (21.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>115.25 (7.41)</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>118.37 (9.02)</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>118.5 (0.7)</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>166 (19.33)</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: STST: Salt taste sensitivity test; SD: Standard deviation.

<table>
<thead>
<tr>
<th>Type</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>40.43</td>
<td>10.85</td>
<td>41.46</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.01</td>
<td>8.5</td>
<td>173.42</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.7</td>
<td>14.83</td>
<td>74.23</td>
</tr>
<tr>
<td>BMI</td>
<td>24.91</td>
<td>4.37</td>
<td>24.57</td>
</tr>
<tr>
<td>Volume of urine (mL/24 h)</td>
<td>1299.33</td>
<td>336.98</td>
<td>1328</td>
</tr>
<tr>
<td>Urine sodium (mg/24 h)</td>
<td>1270.66</td>
<td>326.68</td>
<td>1270.66</td>
</tr>
<tr>
<td>Primary blood pressure (systolic)</td>
<td>112.5</td>
<td>12.98</td>
<td>114</td>
</tr>
<tr>
<td>Primary blood pressure (diastolic)</td>
<td>71.33</td>
<td>10.82</td>
<td>73</td>
</tr>
<tr>
<td>Secondary blood pressure (systolic)</td>
<td>112.83</td>
<td>11.72</td>
<td>113</td>
</tr>
<tr>
<td>Secondary blood pressure (diastolic)</td>
<td>72.16</td>
<td>9.78</td>
<td>74</td>
</tr>
</tbody>
</table>

Note: BMI: Body mass index; SD: Standard deviation.
with urine Na in all individuals \((r=0.93, P<0.001)\), men \((r=0.863, P<0.001)\), and women \((r=0.939, P<0.001)\). Similarly, the correlation coefficients \((P\text{ values})\) for the distance between recognition and tolerance thresholds with urine Na in all subjects, men, and women were estimated as 0.830 \((P<0.001)\), 0.849 \((P<0.001)\), and 0.827 \((P<0.001)\), respectively (Table 3). It should be noted that controlling the effect of gender in correlation models did not change the results. Therefore, gender cannot be considered as a confounder for these correlations.

The determinant coefficient for the recognition threshold, the tolerance threshold, and the distance with urine Na in all subjects were 0.007, 0.86, and 0.69, respectively (Figure 1).

Finally, comparing the correlation coefficients between men and women showed no significant difference between genders regarding the correlations between the recognition threshold \((P=0.082)\), the tolerance threshold \((P=0.668)\), and their distance \((P=0.679)\) with urine Na (Table 3). Regarding the salty taste of the study participants, only five (16.75) of them showed desiring more salts in their diet, indicating that such behavior among our subjects was not considerable for distorting the results of the present study.

**Discussion**

The results of this study showed that STST in the tolerance threshold has a strong and positive correlation with the results of 24-hour urine Na. Our results also demonstrated that a greater distance between the tolerance and recognition thresholds, and a higher urinary Na content. Several methods including 24-hour recall and food frequency questionnaire have been proposed for measuring the amount of salt consumption in the community, all of which are prone to recall bias (2). On the other hand, the 24-hour urine Na test, which shows the amount of salt intake during the last day, is a gold standard and objective method in this context. However, this method is time-consuming, limiting its widespread use in large-scale community studies (18).

The aim of this study was to develop a quantitative taste-test in order to determine the tendency to salt consumption over time which can provide reliable clinical information in less than 10 minutes. This method is low-cost and easy-to-perform in comparison with the 24-hour urine test. In contrast to our results, Kusaba et al found that the results of 24-hour urinary Na excretion were directly correlated with those of recognition threshold for salty taste. However, their study was conducted on patients with chronic kidney disease, which may explain the differences with the present study (1).

Myanmar and Korea reported that the systolic blood pressure was significantly correlated with the results of detection and recognition thresholds, but not with the spot urine Na test (19). Conversely, Kubota et al (20) reported no association between daily salt intake and the salty taste recognition threshold among the healthy Japanese normotensive population. There are some
possible reasons that may justify the differences between Kubota and colleagues’ study results and our findings. First, in contrast to our study, the spot urine collection method was used in the above-mentioned study as the main method for measuring Na. Moreover, Kubota et al. used a salt strip to determine salt taste sensitivity, which is a different result from the current study.

In their study, Lee et al. found that salt taste sensitivity did not reflect the real salt intake in healthy individuals (21). This finding is in line with those reported by Kubota et al., where the spot urine Na method was used to measure Na in the samples.

In our study, determinant coefficients represented that the results of the tolerance threshold and the distance of STST reflect 86% and 69% of changes in the Na content of 24-hour urine samples, respectively. It may introduce STST as a reasonable predictor of Na amounts, which can be replaced with the 24-hour urine test.

In the present study, the correlation between STST results and 24-hour urine Na was not gender-dependent. This is one of the strengths of the present study, which has not been considered in previous similar investigations. Moreover, a maximum tolerance concentration was considered for the salt solution in this study. Following the determination of the recognition threshold, the test was continued to identify the tolerance threshold in order to calculate the distance between these two scores. This is a novel evaluation method, which has not been used in similar studies.

Although it seems that women may be more sensitive to taste than men, the results of our study indicated no difference between genders. Therefore, the hormonal situation can not affect taste recognition.

Limitations of the Study
It should be noticed that the present study had some limitations, especially the small sample size due to problems and the lack of individuals’ cooperation in collecting 24-hour urine. Therefore, the results should be interpreted with caution.

Conclusions
Our study provided reliable evidence regarding the usefulness of STST, especially the tolerance threshold score, as a substitute for the 24-hour urine Na test. However, more studies with higher sample sizes on various populations are recommended to further confirm STST as an ideal method for screening and identifying high-risk populations.

Authors’ Contribution
RM and HEZ, MA, AR: concept and design. RM and HEZ: data collection and interpretation of the data, performing of the study and writing of the draft. All authors read and approved the study

Conflict of Interests
None declared.

Ethical Issues
This study was approved by the Ethics Committee of the Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References