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Pilot Study: A Non-Invasive Urine Test for Potential Prostate Abnormalities

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Abstract: Currently, serum is used more often than urine to detect prostate specific antigen (PSA). The need for a non-invasive test yielding similar results led us to develop a urine test that uses solar irradiated water as a reactant species. To develop this technology, seven reagents plus one control were produced by exposure of water for 40 days in sunlight to the colors of the visible spectrum through colored cellophane, control being an unwrapped bottle of sterile water. Patients (127) were examined for serum PSA and the urine was tested using the above reagents. A positive urine test was observed with yellow-filtered irradiated water which absorbed at 454nm. Twenty-five of the 45 patients with positive results for the urine test had PSA levels of 0.21-4.0 ng/ml. Thus, this pilot study describes a non-invasive urine test mainly positive in patients with PSA 0.21-4.0 ng/ml.

Keywords: Prostate specific antigen, solar irradiation, cancer screening, urine test.

Introduction

Prostate cancer is recognized as the most common type of cancer and the second leading cause of cancer-related deaths in men [1]. The principle screening tests for detection of asymptomatic prostate cancer are the digital rectal examination (DRE) and measurement of the serum marker prostate specific antigen (PSA) [2]. Screening may also include a urine test to check for blood or infection.

The principal drawback of the PSA test is its imperfect specificity, owing to the fact that common conditions, such as benign prostate hyperplasia (BPH) and prostatitis, can cause borderline or even

markedly abnormal test results [3, 4]. These results can lead to expensive diagnostic evaluation and unwarranted patient anxiety. At the other extreme, the high sensitivity of the test can result in over diagnosis. There is always the chance that small, indolent tumors which might require no treatment and which may never have surfaced clinically, would be gathered in the same net and indicated to be aggressive, potentially life threatening cancers [5]. There is also debate over the level of PSA-2.5 versus 4.0 ng/ml-that could be considered abnormal and might warrant biopsy [6, 7]. For this reason, an alternate urine test, which is partially positive with a serum PSA of <4.0 ng/ml, may be useful in detecting a subgroup of patients who do not yet require tumor biopsy.

The rational for choosing the current approach is based on the use of solar irradiated water for healing purposes some dating back to ancient healers and others to a more recent use of solarized water with certain additives [8, 9]. Sun exposure for a minimum of five hours and maximum of 48 hours has been used to decontaminate water from bacteria and viruses [10-15]. Solarization for a minimum of six weeks has also been used to control pest infestation in farming [16]. The biomodulatory effects of individual colors of the spectrum have not been fully characterized.

Our main interest was to develop in-vitro diagnostic testing using sun exposed water. This study investigates the diagnostic effect of mixing urine from urology clinic patients with solar irradiated water. We hypothesize that each spectrum of visible solar light can have diverse influence on different biological entities, and the type of influence may depend on the energy transfer.

To test this hypothesis, we subjected plastic bottled (50 % light transmission) sterile water to sunlight irradiation through violet, indigo, blue, green, yellow, orange, and red colored cellophane paper for a continuous 40-day period in an open environment. The bottles stood in an upright position. Irradiation generated seven reagents corresponding to the visible spectrum of light, along with a control corresponding to an unwrapped bottle exposed to polychromatic light rather than through particular colored cellophane [17]. These energized waters were then used as reagents for a urine screening kit to test abnormalities in the prostate.

The goal of the present study was to assess the potential usefulness of irradiated water in determining the colorimetric changes in the reaction between irradiated water and the urine from patients. For this purpose, the characteristics of the interaction between the urine and monochromatic irradiated waters were first compared with polychromatic irradiated water. The positive colorimetric tests were then compared with the distribution profile of serum PSA. The absorbency profile of a positive reaction was also investigated spectrophoretically.

Materials and Methods

Preparation of reagent water

Sterilized (double-distilled) water in sterilized translucent (50% transparent) plastic (a copolymer of 98% polypropylene and 2% polyethylene) bottles was obtained from Baxter (Deerfield, Illinois). The labels were removed from the bottles. The bottles were then wrapped in cellophane corresponding to

the visible monochromatic spectral colors (violet, indigo, blue, green, yellow, orange, and red). Controls included one unwrapped bottle of sterilized water. The sterilized water in plastic bottles was incubated during the months of October and November in Jackson, Mississippi. The bottles (minimum of two bottles for each coloration were exposed to all daily hours of sunlight for 40 day and were then wrapped in aluminum foil without removing the cellophane and placed in the dark to avoid unwanted light exposure.

Urine test procedure

Urine was collected from patients who visited the urology clinic between the hours of 8.00 a.m. and 12.00 p.m. The first voided urine was collected and dip-test analysis of the urine was performed. Lack of infection was established. None of the subjects tested were under medication. The urine sample was stored at 4^{o}C until it could be processed. The pH of the urine samples was measured again before testing. The pH of the reagent water was ≥5.5. We tested urine samples of pH 6.0 to 6.8 to be within one pH unit difference. Urine samples (100 µl) taken in triplicates were mixed with 100 µl of different irradiated water samples and incubated at room temperature for seven days in a 96-well, flat bottomed tissue culture plate with a low evaporation lid. The first three wells were incubated with the control (polychromatic) irradiated water, followed by three wells of violet, three of indigo, and three of blue in the 1st row of the plate. The second row was incubated with four wells each of green, yellow, orange, and red irradiated waters.

To further characterize the specificity of the reaction, 2 ml of urine was mixed with an equal volume of irradiated water and incubated in a test-tube for seven days at room temperature. The contents were then transferred to cuvettes to determine the absorbency profile at different wavelengths. Healthy male (N=10; median age 33 years) and female volunteers (N=10; median age 36 years) chosen from laboratory and hospital personnel to act as the control group. Normal reactions did not undergo serum PSA evaluation. The urine test using yellow irradiated water observed no detectable color reaction for both the male and female volunteers, except for one male. This individual, who had no disease symptoms, was followed up with a serum PSA test, which was determined as 3.3 ng/ml. We, therefore, excluded this individual from the study.

PSA determinations

Serum PSA was measured with the Hybritech Tandem-R monoclonal radioimmunoassay. This assay was done by an independent urology lab. The results of the urine test were compared with the PSA test. The PSA distribution was arranged in the following categories: <0.1, 0.1-0.2, 0.21-4.0, 4.1-10.0, >10.0 (Table 1).

Table1. Distribution profile of the urine test with PSA measurements.

PSA	(+) Urine	(-) Urine	Total
0	2	6	8
< 0.1	5	19	24
0.1 – 0.2	7	17	24
0.21 – 4.0	25	27	52
4.0 – 10.0	2	6	8
> 10.0	4	7	11
	45	82	127

Results

The average pH of the urines collected and tested was 6.0-6.8. The exposed water had an average pH of 7.0. We wanted to stay within 1 pH unit for the urine test so as to collect data which is not attributed to pH fluctuations. There were two males with PSA 0.21-4 ng/ml whose pH value did not lie between 6.0 and 6.8 that were excluded from the study.

In Figure 1, there is a clear-cut difference between the control (polychromatic irradiated) water and the yellow-filtered irradiated water. Two rows with a positive test corresponding to a darkening of the pigmentation of the urine are in second row. A negative test of the urine is shown in the lower portion of Figure 1. There is some staining that occurs in other than yellow irradiated water as shown in the two strips. The difference in staining between the first 3 wells of the row for each subject and the yellow irradiated water is distinctly different. This test represents only a macroscopic test result as seen by the naked eye.

The observations made in the presence of the control-irradiated water were compared with water exposed to sunlight through different colored cellophane papers; i.e., the polychromatically irradiated water was used as a blank for the spectrophotometer reading. Figure 2 is a representative distribution profile of a reaction between urine and different irradiated waters. There are two peaks and one valley in the absorbency profile. The peaks occur at 397 nm and 454 nm. The valley occurs at 418 nm. Peak at 454 nm corresponds to a reaction specifically between yellow-filtered irradiated water and urine. The urine from normal volunteer gave the same reaction as the control i.e., there was no increase in absorbance at 454 nm implying that a positive reaction with yellow-irradiated energized water is specific.

The positive patient urine tests were then compared with their PSA readings. The results of the PSA readings were arbitrarily divided into five categories as follows: <0.1; 0.1-0.20; 0.21-4.0; 4.0-10.0; and >10.1 ng/ml. A positive urine test was observed in forty-five patients. The majority of positive patients (25 of 45) had a PSA level of 0.21-4.0 ng/ml. Twelve out of 45 had a PSA value <0.21, and six of 45 were above the 4.0-ng/ml level. There were 2 (out of 45) false-positive tests indicated by a negative PSA reading. In the same range of 0.21-4.0 ng/ml PSA, there were 27 out of 52 false-negative tests.

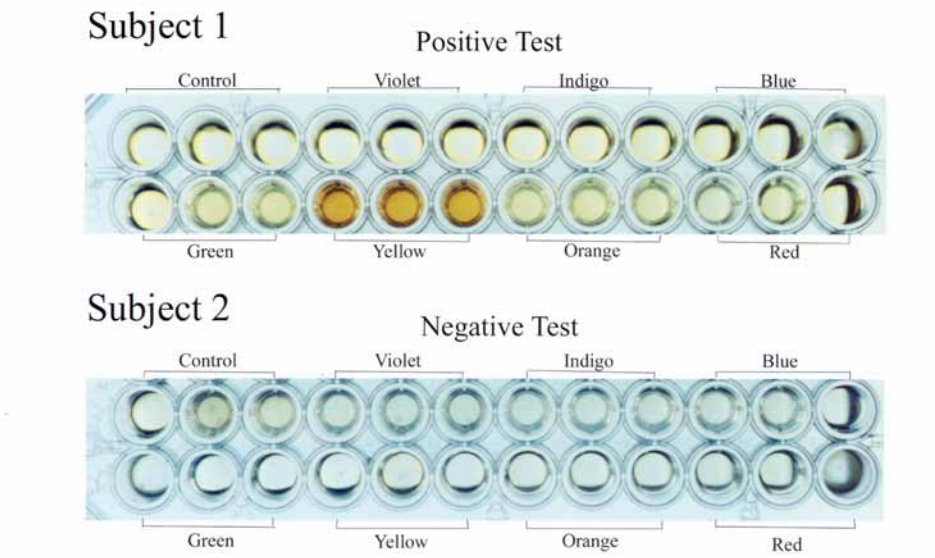

Figure 1. A positive and negative urine test in a 96-well plate from subject 1 and 2. The urine samples (100 µl) in triplicate, were mixed with equal volume of solar exposed water i.e., in the first three wells of row 1 control (exposed water in unwrapped bottles), the next three wells with water exposed through violet cellophane paper, followed with three wells of indigo cellophane exposed water and the last three wells with blue cellophane exposed water. The second row consists of urine and green exposed water in the first three wells, the next three wells contains yellow exposed water and the next three contain orange and the last three wells contain red exposed water. Note a positive test with urine and yellow irradiated water. The negative test as shown occurs in the last two rows where the urine mixed with yellow exposed water gives no reaction.

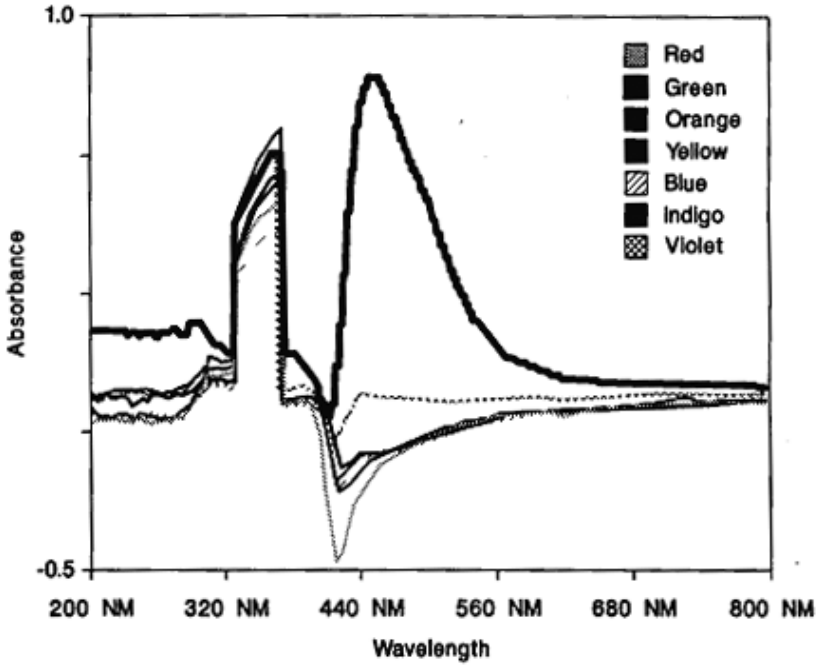

Figure 2. Optical density profile of urine samples with water exposed to the different colors of the solar spectrum. The patient's positive urine reaction with the yellow exposed water gives a unique absorption peak at 454nm. The patient's PSA serum level was in the range 0.21-4.0 ng/ml.

There was no reaction in urine of normal women (N=10; median age 36 yrs), nor in urine of normal males (N=9; median age 33 yrs) using the yellow-filtered irradiated water.

Discussion

The serological determination of PSA is widely accepted to be the best method for screening, diagnosis, and follow-up in prostate cancer. PSA is not only present in the serum but also in other body fluids such as urine and semen specimens [18, 19]. This pilot study describes a new non-invasive method as an alternative for screening and perhaps diagnosing prostate cancer. The color change of the patient's urine when mixed with, monochromatic (574 nm) solar irradiated water is being considered to indicate prostate abnormality. Further, the results from the urine sample are compared to the serum PSA levels of the subject. The data indicates that of the seven monochromatic and the polychromatic irradiated water only the yellow one reacts partially (48% mean sensitivity) in subset of patients with serum PSA ranging from 0.21-4.0 ng/ml. Current methods of detection are not cost-efficient and reported values for the sensitivity (29-80%), specificity, and positive predictive values of PSA may not reflect true values [20].

The results reported in this investigation are a pilot study in using irradiated water as an alternative testing system. The first step in developing a urinary testing kit for the detection of prostate abnormality in our investigation was to determine if there was any unique reaction between the urine and the irradiated waters. A unique color reaction was observed when the urine reacted with yellow-filtered irradiated water. The fluid in the wells became dramatically deeper in pigmentation. It is noteworthy that the irradiated water *was not colored yellow, but was irradiated by sunlight through yellow colored cellophane* for 40 days. Urine normally has more or less intense yellow color, but the nature of the substances (urochromes) responsible for this is unknown. Thus, the chemical change that took place is due solely to a reaction between the urine and the water irradiated at 565-575 nm. This change in coloration was considered to be a positive urine test. There is an inherent control in the design of this test, because all three wells gave a similar reaction. This reaction was further tested in the presence of urine from healthy females and healthy males whose results were negative. A positive urine test, using undiluted urine was observed in 45 patients; of that, 25 patients had a total PSA of 0.21-4.0 ng/ml.

One of the main drawbacks of this pilot study is the use of a macroscopic test. The decreased sensitivity (48%) of the test may be based on the decreased sensitivity of the visual examination. The next phase of experimenting would employ both the macroscopic test and the utilization of a plate reader to read the absorbance at 454 nm. The utilization of spectrophotometric quantitation should improve sensitivity of the method. Also, the controls would have both the urine and blood analyzed to determine the actual PSA value of the individual tested rather than assuming that the normal healthy individuals in the population have <0.1 ng/ml PSA.

In our lab studies, we have previously observed evidence of alterations in chemical and physical properties of water and biological functions by using colored cellophane during sun exposure (unpublished observations). Water exposed (E) to visible spectral emissions of sunlight was found to have an altered elemental composition, electrical conductance, osmolarity and salt-solubility. A difference in bio-modulatory effects was also observed. A gradual increase in leaching of Boron from E-violet to E-red was observed. The maximal increase in electrical conductance and maximal salt solubility of sodium bicarbonate was found with E-indigo. E-blue inhibited phyto-hemagglutinin-induced immune cell proliferation and inhibited mosquito larvae hatching, while E-orange stimulated root elongation in seed germination. A point to note is that solarization has been used to decontaminate drinking water which is based on the exposure of living organisms (bacteria and viruses) to UV-light and possible thermal inactivation. However, in our study sterile water has been exposed to sunlight irradiation for a long period of time (40 days), a procedure that clearly has a different mode of action compared to disinfection.

In the pilot study presented here, we have clearly shown that there is a chemical change in the presence of yellow-filtered irradiated water manifested by a change in the coloration of the urine with an absorbance peak at 454 nm. "Energized water" described in this study at specific wavelength of the visual spectrum will interact with the urine from patients with prostate abnormality is by itself an intriguing observation. One may consider that energized photons at this wavelength have sufficient energy to move the hydrogen and oxygen electrons in higher orbitals. If so, when the "energized water" is mixed with the urine, these electrons return to their original orbitals, emitting photons which are absorbed by whatever substance (urochromes) are in the urine-producing the described color change. Further, solar irradiation might have produced oxygen reactive species (by photolysis) in the water such as superoxide O_{2-}, hydrogen peroxide H_2O_2, hydroxyl radical $OH\cdot$ (by Haber Weiss reaction; $O_{2-} + H_2O \rightarrow OH\cdot + OH^- + O_2$) and by spontaneous dismutaion of the superoxide O_{2-} to singlet oxygen $^1O_2\cdot$. However, the end-product of the reactive oxygen stress is a chemical reaction which involves hydrogen peroxide, a bleaching agent, which would not augment the color reaction in the positive urine test we observed but would instead give a dimunition in the color change which is contrary to what was empirically observed. A point to note in the 96-well plate is that the absorbance peak was at 454 nm, while the color was macroscopically observed, i.e., the yellow pigmentation corresponds to the emission spectra in the yellow color range (565-575nm).

In this study, a visual change in the indicator system was considered optimum. The specifics of the changes in the colorimetric reaction were determined by evaluating the optical density in a spectrophotometer. It is clear that urine is carrying the causative agent for the color reaction while the irradiated water carries the antidote for this agent. A color reaction takes place because of the attraction or interaction of a component or components in the urine with the irradiated water. The mechanism of action between the yellow-filtered monochromatic irradiated water and the urine, however, is not completely understood. Some trace amounts of proteins, hormones and other substances normally found in urine may potentially interact with solarized water. It is not clear whether the color reaction observed was a simple reaction between the dye constituting the pigment in the urine or some

combination of secreted protein and dye. It is possible that the sex hormones, like testosterone, may interact with the pyrrolic ring structure present in the pigment. One of the ways to test this premise would be removing the pigment by acetone treatment or by passing the sample over activated charcoal to study the individual components or their combination for positive reaction with yellow filtered irradiated water sample. This test predominantly detects a subset of patients whose PSA is in the range of 0.21-4.0 ng/ml.

It is possible that the urine test may be different for different times of the day. In order to minimize these variations, 24-hour urine samples must be collected and analyzed. Since the prostate is a testosterone-dependent organ it is possible that urine tests could be negative due to the patient's having received a treatment that alters hormone levels (castration or diethylstilbestrol). Along the same lines, testing women's urine must include pre- and post-menopausal samples. Another important point to consider is the relationship of PSA in urine and serum. High levels of PSA in serum are suggestive of metastasis while PSA in the urine may indicate either the presence or absence of disease. Additional urine PSA testing should be done to determine whether there is any correlation between urine PSA and the positive results in the urine test presented here. Also it would be worthwhile to correlate the in-vitro results (positive or negative) with clinical history of patients.

In our study, we have developed a new innovative test that is easy to use, involves different biochemical assays than antibody mediated PSA tests, and uses urine instead of serum. Urine is more easily obtained and disposed of and less risky to handle than blood products. For the patient, the use of urine instead of blood eliminates the necessity of an invasive procedure and its possible complications. The ingredients of the kit, i.e., urine, yellow-solar-irradiated water, and a 96-well plate are environmentally friendly. The innovative technology is the transfer of solar energy into the bottled water. This technology is natural, simple, and has never been employed for *in vitro* diagnostic testing.

In conclusion, we present evidence here that a potential new urine test has been developed that is negative in women and healthy males but is positive in a subset of patients, especially in those patients with serum PSA 0.21-4.0 ng/ml. Additional studies are needed to determine the specificity and sensitivity for this assay.

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