Clinical Research

A study on the method of Taila Bindu Pariksha (oil drop test)

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Abstract

Taila Bindu Pariksha, an ancient method of urine examination for ascertaining the prognosis of diseases, was very popular in the medieval period, the use of which became obsolete after 17th Century AD. Technique of this test is very crude and there are chances of variations in the observations. To revive this technique, it is necessary that the methodology of this test should be standardized so that the observations could be reproducible. To standardize the technique, apparently healthy volunteers were selected and various parameters were standardized for doing this test, i.e., shape and size of Patra (testing containers), volume of the urine, size of the oil drop, height of the oil drop from the surface of urine, variety of sesame oil, etc. Based on the literature, the parameters were changed one by one and observations were noted down. The whole method was recorded in the form of video clips for proper evaluation. The parameters standardized on the basis of observations can be considered as standard to be referred in future studies.

Key words: Oil, standardization, Taila Bindu Pariksha, urine

Introduction

Taila Bindu Pariksha is described in number of Ayurvedic texts like Vangasena Samhita,[1] Vasavarajiyam,[2] Yogaratnakar,[3] Yogatrangini,[4] etc.. It was very popular in the medieval period, but after 17th century AD its use became obsolete.[5] In Taila Bindu Pariksha, urine is taken in a glass vessel over which an oil drop is dropped and behaviour of oil is noted down. The features (oil over floating water) are indicative of prognosis of diseases.

On critical analysis, it can be observed that there are variations in the parameters related to this test. Hence, this study was undertaken with the aim to standardize these variables.

Materials and Methods

Standardization of “Tila Taila”

In Ayurvedic texts, there is no reference depicting whether black or white variety of sesame (Tila) should be taken for “Taila Bindu Pariksha.” In the present study, we decided to compare various physical and chemical properties of both varieties and an attempt was made to see whether the behaviour of oil in urine is affected by the use of both varieties of Tila or not. With this aim, three samples of each white and black variety were taken from different suburban parts of Uttar Pradesh. These samples were identified by the experts of Dravyaguna, and after identification, samples were numbered as: W1, W2, and W3 – white series (white Tila seeds); B1, B2, and B3 – black series (black Tila seeds). Oil was extracted and stored in glass containers. Thin layer chromatography (TLC), specific gravity, viscosity, saponification value, and preliminary phytochemical studies for all the samples were done. Three solvent systems were used in TLC as mentioned below:

1st: Benzene–petroleum ether (1:1)
2nd: Pure benzene
3rd: Benzene–chloroform (8:2)

Reagent used was Liebermann reagent.

Standardization of method of Taila Bindu Pariksha

In standardization of the procedure, it was planned to standardize the following variables of Taila Bindu Pariksha.

- Shape and size of Patra (testing containers)
- Volume of the urine
- Size of the oil drop
- Height of the oil drop from the surface of urine
- Duration between collection and performance of test
- Type of Tila Taila for test

Parameters observed during Taila Bindu Pariksha

- Shape of oil after spread
- Direction of spread
- Spread time and spilt time
- Area covered
Standardization of the above variables was done on apparently healthy volunteers of either sex between 20 and 30 years of age with no drug history at least for 1 week and those having similar diet pattern on previous day of the test. Urine routine and microscopic examination was done and cases with any abnormality were discarded for the present study. Video recording of the whole procedure was done to provide evidence.

The difference in constituents of urine depends upon the dietary metabolites and other minor constituents which are excreted through urine. Presuming the interference of the dietary metabolites with the behaviour of oil drop in urine, apparently healthy volunteers who stayed in the hostel were selected as the hostellers have fixed menu in the diet. For standardization, the test should be performed in constant parameters, so it was planned that one parameter will be changed at a time and any difference in the observations will be noted down. For getting finalized standard parameters, the following parameters were taken for the study.

**Material of Patra (testing container)**
Almost all Ayurvedic texts have instructed to use glass container for Taila Bindu Pariksha except Vangasena who has stated that either glass or bronze can be taken. Glass was chosen as standard in the current work on the basis that cleaning and availability of glass vessel is easier and glass is cheaper than bronze.

**Shape of Patra**
Test was performed in square and round shaped vessels of the same dimensions and observations were noted down.

**Size of drop**
In Ayurvedic literature, Trina was used as oil dropping media over urine surface. Average weight of oil drop was calculated and then volume of one drop was determined by following formula:

\[ \text{Volume of one oil drop} = \text{average weight of one oil drop/density of oil.} \]

**Volume of urine**
Test was performed after thorough mixing of urine sample using different volumes of the same sample, keeping the other parameters constant. Volume of urine sample was changed each time. Test was performed using 200 ml, whole voided sample, and the amount which covered at least three-fourths of the testing container.

**Size of testing container**
Keeping other parameters constant, Taila Bindu Pariksha was done by dividing the same urine sample and putting it in different sizes of testing containers having 4 inch, 6 inch, and 8 inch diameter.

**Height of the oil drop from the surface of urine**
This was done by dropping the oil from the height of 10 cm, 5 cm, and 1 cm from the surface of urine.

**Time of Taila Bindu Pariksha**
Time factor (i.e., time between collection of urine sample and performance of test) was another variable for standardization. For this, recording of observations of the same urine sample was done immediately after collection, at a time gap of 2 h after collection, and at a time gap of 5 h after collection.

**Variety of sesame oil**
For selection of oil, urine sample was divided into three parts and Taila Bindu Pariksha was performed by oil of white series (W₁, W₂, and W₃) and black series (B₁, B₂, and B₃) one by one.

**Results**

**Standardization of Taila Taila**
The observations of TLC reveal that different samples of the same variety of sesame contain identical compounds as substances having the same Rf value and are likely (but not necessarily) to be the same compound (Tables 1 and Figures 1-6).

Specific gravity and viscosity of the black variety was found to be slightly greater than those of the white variety. Due to these properties, the black variety was taken for this study as it will provide time to observe the pattern as compared to the white variety. Among the types of black variety, B₃ has the highest viscosity (hence slower spread), considering which the same was selected for the study and used as standard oil.

Preliminary phytochemical study of the samples revealed the presence of steroids, reducing sugar, and terpenoids in all the samples. It was matched with the data reported in earlier studies.

**Standardization of Taila Bindu Pariksha procedure**

**Shape of testing container**
In this study it was observed that the shape of testing container does not affect the spread of oil drop. As availability of round vessel was easier, it was chosen as standard for this study.

**Size of drop**
The average volume of one drop came to be 12.48 μl (Table 2). A round figure of 12 μl was taken as standard for this study. As it was not possible to get Trina of the same size throughout the study period, Pasteur pipette or micropipette of the same caliber was used in the study.

**Volume of urine**
The observations were almost same in three volumes tried – 200 ml, whole sample, and urine volume up to three-fourths of the Petri dish (Tables 3-5). It was decided that whole voided sample will be taken as it will provide a chance to have maximum number of surface active molecules so that accurate observations can be obtained even if the number of surface active molecules is less.

**Size of Petri dish**
Petri dishes of 4 inch, 6 inch, and 8 inch diameter were tried. Observations on different Petri dishes are tabulated (Tables 6-8).

### Table 1: Observations of various tests done on six samples of Taila Taila (sesam oil)

| Tests performed | B₁ | B₂ | B₃ | W₁ | W₂ | W₃ |
|-----------------|----|----|----|----|----|----|
| Specific gravity| 0.96| 0.96| 0.96| 0.95| 0.95| 0.95|
| Viscosity (in centipoise) | 63.25| 62.92| 60.20| 60.20| 60.76| 60.52|
| Saponification value | 180.92| 169.70| 232.81| 218.79| 200.56| 193.55|
Table 2: Size of drop

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Weight of 1 drop of oil/ density of oil | 0.012/0.96 | 0.011/0.96 | 0.013/0.96 |
| Volume of one drop of oil | 12.50 µl | 11.45 µl | 13.5 µl |

Table 3: Volume of urine: With 200 ml of urine

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 25 sec | 28 sec | 30 sec |
| Split time | 90 sec | 100 sec | 105 sec |

Table 4: Volume of urine: With whole sample

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 32 sec | 35 sec | 30 sec |
| Split time | 96 sec | 120 sec | 105 sec |

Table 5: Volume of urine: Up to three-fourths of Petri dish

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 34 sec | 38 sec | 32 sec |
| Split time | 110 sec | 120 sec | 100 sec |

Table 6: Observations on Petri dish of 4 inch diameter

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 75 sec | 50 sec | 60 sec |
| Split time | 180 sec | 210 sec | 160 sec |

Table 7: Observations on Petri dish of 6 inch diameter

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 50 sec | 40 sec | 55 sec |
| Split time | 140 sec | 120 sec | 140 sec |

Table 8: Observations on Petri dish of 8 inch diameter

| Observations | Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|--------------|
| Shape | Circular | Circular | Circular |
| Spread time | 30 sec | 36 sec | 34 sec |
| Split time | 90 sec | 106 sec | 78 sec |

From the observations [Figures 7-9], it was inferred that Petri dish of 8 inch diameter provided minute details of shape as well as comfortable spread and split time. So, it was chosen as the standard Patra for this study.
from 5 or 10 cm, waves were produced on the urine surface, resulting in distortion of shape of oil film [Figures 10 and 11]. For proper observation of shape, it was necessary that urine surface should be calm and quiet, so height of 1 cm was chosen as no waves were observed. Hence, this height was set as a maximum height from which oil would be dropped.

**Time of Taila Bindu Pariksha**

Taila Bindu Pariksha was performed at three different timings – just after collection, within 2 h, and after a gap of 5 h [Table 9 and Figures 12-16]. There was major differences in the spread and split time under these three observations. Since the rate of spread was neither fast nor slow when performed within 2 h of collection and it testifies the textual description, a gap of 2 h between collection and performance of test was taken as the standard time for performance of test.

### Discussion

**Material of testing container**

As minute traces of lipid or any surface active molecules that can affect the spread of oil film can be easily removed from a glass vessel and as glass can be washed with very strong acid or alkali, glass vessel was preferred over bronze. Secondly, glass vessel is cheaper than bronze and also its easy availability justifies its preference as the material of testing container.

**Table 9: Time of Taila Bindu Pariksha**

| Observations | Immediately after collection | At time gap of 2 h | At time gap of 5 h |
|--------------|-----------------------------|-------------------|-------------------|
| Shape        | Circular                    | Almost circular   | Circular          |
| Spread time  | 105 sec                     | 40 sec            | 3 sec             |
| Split time   | 180 sec                     | 110 sec           | 9 sec             |
the rate of spread was neither fast nor slow when performed within 2 h of collection and it testifies the textual description, a gap of 2 h was kept as the standard between collection and performance of test.

**Variety of oil**
As spread in black variety was slow in comparison to that in white variety due to more specific gravity and viscosity, it allowed sufficient time to observe the behaviour of oil. Since B$_2$ sample was having higher value of specific gravity and viscosity, it was decided that B$_2$ will be used as standard oil in this study.

**Hypothesis regarding the interaction between the molecules present in the urine and the oil**
The interaction between the urine molecule and the oil in Taila Bindu Pariksha can be explained on the basis of the mechanisms of oil spill on sea surface. There are three stages in the spread of initially concentrated volumes of oil on a calm sea. Immediately after the spill, the oil slick is rather thick. Therefore, in the first phase, gravity and inertia forces dominate the spreading process with gravity being the accelerating force and inertia the retarding force. As time progresses, the oil slick becomes thin and inertia forces become relatively unimportant. In the second phase, the gravity and viscous forces dominate the spreading with the viscous force being the retarding one. As the slick gets thinner, interfacial tension forces become important. A third phase is reached in which interfacial tension and viscous forces dominate the spreading. In case of Taila Bindu Pariksha, the first phase is not important as weight of oil drop is very less and thus effect due to gravity is negligible in this case. In Taila Bindu Pariksha, the third stage may be the final determinant of the extent and rate of spread.

Regarding the various patterns of spread of oil on the urine, as a hypothesis it can be said that the surface active molecules and other metabolites present in traces probably affect the spread. These are substances which are normally not recordable and they determine the spreading pattern of oil. The directional spread of oil may be explained on the basis that there may be presence of the paramagnetic molecules which may align as per the magnetic field of the earth, giving a directional spread to the oil. The interfacial tension between the surface active molecules and the oil may decide providing possibilities of different shapes, speed, and extent of spread.

**Conclusion**
The parameters which were standardized need further evaluation in more number of samples to establish the above observations. Some of the important physical parameters like viscosity of the urine and interfacial tension could not be measured due to unavoidable technical constraints. The viscosity and interfacial tension may show some relation with spread, split time, etc., Finally, it may be concluded that Taila Bindu Pariksha can be used as a tool for assessing the prognosis and severity of diseases to plan the treatment. This simple technique may also be helpful in diagnosis of diseases as well as in assessing the healthy conditions. But it requires observations in large number of cases. Since no laboratory test is available to instantly assess or forecast the prognosis of the diseases, this method which is very cost effective may be proved to be a useful technique in this field.

**Shape of testing container**
In this study, it was observed that shape of testing container does not affect the spread of oil drop. As availability of round vessel was easier, it was chosen as the standard for this study.

**Volume of urine**
The observations were almost the same in the three volumes tried, i.e., 200 ml, amount which covers three-fourths of the Petri dish, and whole voided sample. It was decided that whole voided sample will be taken on the basis that the above condition will provide a chance to have the maximum number of surface active molecules and so accurate observations can be obtained even if the number of surface active molecules is less.

**Size of Petri dish**
Petri dishes of 4 inch, 6 inch, and 8 inch diameter were tried. The shape in all Petri dishes was the same, but differences in spread time, split time, and area were observed [Figures 7-9]. Presence of surface active molecules over which oil cannot spread is expected to restrict the extent of spread depending on the quantity of such molecules, and therefore in smaller dishes, the diameter of the spread will be less. This was demonstrated in the experiment with different sizes of the Petri dishes. Due to small area, probability of missing details of oil margin became more. Petri dish of 8 inch provided minute details of shape as well as comfortable spread and split time. So, it was chosen as the standard container for this study.

**Height of the oil drop from the surface of urine**
Heights tried were 1 cm, 5 cm, and 10 cm. As stated in the observations, in the latter two heights, waves were produced [Figures 10 and 11]. For proper observation of shape, it was necessary that urine surface should be calm and quiet, so height of 1 cm was chosen where no waves were observed. Hence, this height was set as the maximum standard height from which oil would be dropped.

**Time of Taila Bindu Pariksha**
Spread was slow when just voided sample was used, and when sample kept for 5 h was used, spread was very fast. Immediately after voiding, value of surface tension of urine was maximum which decreased with time.$^{[39]}$ This might be the reason of slow spread in the starting. Later on, changes occur due to bacterial decomposition of urine constituents, so spread occurs faster. The spread was neither fast nor slow when the test was performed on the samples within 2 h of collection. As said in the texts that the sample should be collected before $4$ Ghatika of sunrise, which on calculation comes to approximately 1 h 36 min, the test should be performed after sunrise. Since
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