



Research Article

STANDARDIZATION OF NEIKURI (OIL DROP TEST OF URINE)– A SIDDHA DIAGNOSTIC METHOD

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ABSTRACT

Neikuri is an antique method of urine examination, based on distribution of oil drop in urine which is a remarkable diagnostic and prognostic tool in Siddha system of medicine. Even though these diagnostic method not commonly used in clinical practice and elaborate scientific studies of *Neikuri* are lacking. **Aim:** The aim of the study was to test the protocols of the examination of *Neikuri* for the standardization. **Materials and methods:** 100 patients were selected and studied with twelve parameters to test the *Neikuri*. Correlation was considered between the reading of *Neikuri* and *Naadi*. **Results:** Based on the observation, the urine needs to place in a circular glass utensil (3.5 inch petri dish preferable) and a drop (0.25 - 0.5 μ L) of sesame oil derived from black sesame seeds need to placed less than 2mm height from the surface of the urine, in day time to test the *Neikuri*. Further the test needs to perform within one and a half an hour from the collection of urine. **Conclusion:** In conclusion we suggest the methods could be used as a standard protocol for testing *Neikuri*.

KEYWORDS: *Neikuri*, Siddha, Standardization, *Naadi*, Diagnostic tool.

INTRODUCTION

The Siddha system of medicine is one of the antediluvian traditional system of medicine in the world and has unique methods for diagnosis of the diseases.^[1] *Neikuri* is the method of urine examination, based on distribution of oil drop in urine which is a remarkable diagnostic and prognostic tool and well explained by sage Theriyar and Agasthiyar.^[1,2] Diagnose of disease using *Neikuri* considered as a special method of confirmation technique than *Envagaithervu* (*Nadi*, *Sparism*, *Naa*, *Niram*, *Mozhi*, *Vizhi*, *Malam* and *Siruneer*).^[1] Spreading of oil on the surface of urine and appearance of the shape of *Neikuri* varied according to the disease condition. In addition to that the *Neikuri* forecast the curable and incurable disease conditions.^[1,2]

The procedures of investigation of the *Neikuri* were documented in text books in the format of Tamil stanza. Early morning urine need to collect into a glass utensil, when the person ingest six variety of taste of food in the previous day night and thereafter following a good sleep. Apply a drop of oil on the surface of the urine, within one and a half an hour from the collection of the urine. The utensil needs to keep in a place away from wind to test *Neikuri*. Further the literature *Noinalad Noi muthal naadal thirattu* has explained that the rules not need to

follow when examine the *Neikuri* to a patient.^[1] In addition the literature *Sikichcharathna theepam* reveals that the *Neikuri* need to test in sunlight using either a glass or clay pot.^[2] Variations observed in the procedures of *Neikuri* examination among the literature in the following parameters; type of the oil used, amount of oil drop, size, material and shape of the utensil used to test the *Neikuri*, volume of urine, collection time of urine, time duration between collection of urine and performance of the *Neikuri* and distance of the placement of oil from the urine surface.^[3,4]

In Ayurveda system of medicine, one of the diagnostic tools called *Taila bindu pariksha* is resemble to *Neikuri*. A previous study documents the standardization of the *Taila bindu pariksha* using the parameters as, type of oil, size, shape and material of the utensil, volume of the urine, collection time of urine and dropping height from the surface of urine.^[3] In addition to that another study confirms the duration between collection of urine and performance of the test of *Neikuri*.^[4]

User friendly modern scientific technologies and a little usage of Siddha diagnostic methods have led to non-familiar of Siddha diagnostic methods in the healthcare and scientific communities. A little brief studies noticed in standardization of *Neikuri*.

Although elaborate studies on standardization of *Neikuri* has not been studied previously. It is essential to give a scientific validation to the methods of testing *Neikuri* for standardize the protocol to the effective reuse of *Neikuri* in clinical practice. Therefore the study was design to test the methods of testing *Neikuri* to develop the protocol for testing the *Neikuri*.

MATERIALS AND METHODS

Selection of patients

The patients above 10 years old^[1] (n=100) in both sex with various diseases (*Vatham*, *Piththam*, *Kabham* and *Thontha* - combine of two *Dosha*) were selected randomly from outpatient department (OPD) and inpatient department (IPD) at Government Siddha Medical College, Palayamkottai, Tamil Nadu.

Standardization procedure and testing the *Neikuri*

Twelve parameters were tested for standardization of *Neikuri*; variety of oil, collection time of urine, time duration between the collection of urine and perform the test, time duration for examine the *Neikuri*, material, colour, size and shape of the utensil used, volume of the urine to test *Neikuri* with the size of utensil, volume of oil drop, distance of the placement of oil from the urine surface and status of the utensil (open or closed chamber). *Naadi* (pulse examination) was observed and recorded while performing the test of *Neikuri* to compare and confirm the *Neikuri*.^[5]

The urine was collected in clean utensils. A drop of oil was placed over the surface of the urine at the centre of the utensil. The nature of spread and formation of shapes and disappear the scatter of *Neikuri* were observed and recorded. Fifteen samples of urine were collected and performed *Neikuri* to test each parameter. All tests were performed in triplicates.

Variety of the oil used

Five varieties of sesame oil (*Sesamum indicum* L.) such as sesame oil derived from black sesame seeds, white sesame seeds, brown sesame seeds and mixture of the above three sesame seeds (60% black, 20% white and 20% brown sesame seeds) were obtained by using mechanical cold press method, at local industries at Tirunelveli District (above described four varieties of sesame oils prepared with adding jaggery). In addition to the above oils, the oil derived from black sesame seeds without adding jaggery was purchased (mechanical cold press method) at Selam District. Urine samples were equally divided in to five similar size, material and

shape utensils separately and *Neikuri* tested. The changes were observed and recorded.

Collection time of urine

Urine samples were collected at morning (first urine, before and after the breakfast), noon and night from the same patients and *Neikuri* tested.

Time duration between collection of urine and perform the test of *Neikuri*

Neikuri was performed, as immediate as collection of urine and after one and a half an hour^[1] from the collection of urine.

Time duration for the examine the *Neikuri*

The time was recorded from as immediate as placed the oil to till the end of spreading, cessation of formation of shape or scattered the oil when testing *Neikuri*.

Material and colour of the utensil to test *Neikuri*

Collected urine (collected once from a subject) was divided in to eight equal volumes and dispensed into various material of containers as glass, plastic and clay pot individually. Various colours (white, yellow, blue, green, black and red) of plastic containers were also used to test the *Neikuri*.

Size of the utensil

Neikuri was tested when using various diameters of utensils such as 2 inch (5cm), 3inch (7.6cm), 3 ½ inch (8.9cm) and 4 inch (10cm) with ½inch (1.3cm) height.

Shape of the utensil to test *Neikuri*

Equal volume of urine (derived from a subject at once) was placed in to circle, square, oval, triangle and irregular shape of utensils, glass bowl and petri dish individually and *Neikuri* tested.

Volume of the urine with size of the utensil

The urine sample derived from a subject was divided into different volumes as 10ml, 20ml, 30ml, 40ml, 50ml, 60ml, 70ml and 80ml. The divided samples of urine were transferred into the petri dishes with various diameters as 2 inch (5 cm), 3inch (7.6 cm), 3 ½ inch (8.9 cm) and 4 inch (10 cm) (large volume of urine was added only into appropriate diameter of petri dishes). The *Neikuri* was tested and recorded.

Volume of oil drop

The urine was divided into four equal volumes and placed into similar size and shape of utensils. Various volume of oil drop (10µL, 20µL, 25µL, 50µL and 100µL) was placed using micropipette and reading recorded.

Height of the placement of oil from the urine surface

The oil was applied from various heights (very closed to surface of the urine, 0.5cm, 1cm, 2cm and 4cm) from the surface of the urine and the results observed and recorded.

Status of the utensil

Urine samples were divided into two equal volumes and dispensed into similar size utensils. Oil was applied into both samples at same time. Lid of the one utensil closed as immediate as applied the oil and the other utensil kept open. The changes observed and recorded.

RESULTS

The result of the tested twelve parameters of testing *Neikuri* has given below.

Variety of the oil

Higher significant association was observed between *Neikuri* and *Naadi* in the sesame oils derived from the black ($F_{1,15}=0.00$, $p<0.001$) and mixed ($F_{2,15}=28.60$, $p = 0.001$) sesame (*Sesamum indicum* L.) seeds. Neither significant association nor correlation was observed between readings of *Neikuri* and *Naadi* in the sesame oils derived from white, brown sesame seeds and oil prepared without adding jaggery. The

spreading was not observed in 86.5% of urine samples when used the oil derived from white sesame seeds.

Collection time of urine

Higher significant association ($F_{2,15}=172.3$, $p<0.001$) was observed between *Neikuri* and *Naadi* when the urine collected and tested at morning and also a significant association ($F_{2,15} = 5.858$, $p=0.012$) was observed with *Naadi* and *Neikuri* when the urine collected and tested at the day time (before sun set). Even though the *Naadi* does not significantly associated with *Neikuri*, when the urine collected and tested at night. No significant different was observed when tested the *Neikuri* using the urine that collected before and after the breakfast.

Time duration between collection of urine and perform the test of *Neikuri*

The mean time taken to start and disappear the scatter of the *Neikuri* has shown in Table 1. Minimum mean time, 153.1 ± 149.7 and 912.1 ± 389.3 seconds were recorded to start (appear of 1st dot) and disappear the scatter of the *Neikuri* (oil drop) respectively, when using the oil derived from black sesame seeds.

Table 1: Mean value of time taken to start and disappears the scatter of the *Neikuri* with variety of oils

Variety of sesame oils (oil derived from different types of sesame seeds)	Time taken to start the scatter (seconds)	Time taken to Disappear of scatter (seconds)
Black	153.1 ± 149.7	912.1 ± 389.3
Brown	1837.6 ± 903.9	1879.3 ± 587.9
White	No spreading	No spreading
Mixture of these three seeds	323.9 ± 334.1	1228.2 ± 582.9
Oil without adding jaggery	233.8 ± 238.8	1677.3 ± 824.9

Duration for examine the *Neikuri*

Higher significant association ($F_{1,15} = 9.024$, $p< 0.001$) was observed with *Naadi* when the *Neikuri* examined within one and a half hour from the collection of urine. The spreading of *Neikuri* was either fast (33.3%) or slow (66.6%) when the *Neikuri* was examined after one and a half hour from the collection of urine.

Material, colour and size of the utensil to test *Neikuri*

Table 2 has shown the association between the time taken to start and disappear the scatter of *Neikuri* with the material and the size of the utensil used for the test. Significant variation observed in time taken to start, spread (formation of shape) and disappear the scatter of the *Neikuri* with the different material and size of utensils used. The minimum time was taken to start and disappear the scatter of *Neikuri* when using clay utensil. However the *Neikuri* does not associated with *Naadi*. The *Neikuri* was significantly associated with *Naadi* in glass utensil (3, 3.5 and 4 inch petri dishes). Even though there was no scatter was observed when test the *Neikuri* using 2 inch petri dish (the oil drop was not spread (73.3%) or a mild spread and it became as a drop after 1502.3 ± 245.3 seconds (26.7%)).

Table 2: Mean value of time taken to start and disappear the scatter of the *Neikuri* with material and the size of the utensil

Material of the utensil	Mean Time taken to start the scatter (seconds)	Mean Time taken to disappear the scatter (seconds)
Glass (petridish) – diameter 2 inch	No spreading	1502.3 ± 245.3
3 inch	448.3 ± 316.8	1123.3 ± 436.8
3.5 inch	130.5 ± 74.8	956.8 ± 467.1
4 inch	201.7 ± 85.2	1212.5 ± 94.5
Plastic	407.5 ± 172.8	1355.0 ± 106.9
Clay	84.25 ± 26.8	694.0 ± 273.4

Table 3 has shown the visibility (%) of *Neikuri* when used different materials and colours of the utensils for the study. The spreading clearly observed in all samples of urine tested (urine colour differed from transparent to dark) in the glass utensil (colourless) and clay pot by unaided eye, than other utensils used for the study.

Table 3: Visibility of *Neikuri* with different materials and colours of utensils used

Material and colour of utensils	Spreading observed by unaided eye (%)
Glass (colourless)	100
Plastic	
White	46.7
Yellow	60
Blue	80
Green	66.7
Black	40
Red	66.7
Clay pot	100

Shape of the utensil

There was no significant variation observed in time taken to start, disappear the scatter of the *Neikuri* and formation of shape with the different shape of utensils (circle, square, oval, triangle and irregular shape). However shape of the spreading of the *Neikuri* easily observed and identified when used circular shape utensil than the other shape of the utensils. Further the changes of *Neikuri* easily observed in petri dish when compared with glass bowl.

Volume of the urine with size of utensil

Table 4 has shown the association between the volumes of urine taken and different size of the utensils. *Neikuri* was observed in tests and no significant variation observed in *Neikuri* when using different volume of urine with different diameter of utensils.

Table 4: Time taken to test *Neikuri*, when using different volumes of urine and different sizes and quantity of utensils

Diameter (inch)	Volume of urine (ml)	Correlation between <i>Neikuri</i> and <i>Naadi</i>
3	30	$p < 0.041$
3 ½	50	$p < 0.001$
4	80	$p < 0.01$

Volume of oil drop

Table 5 has shown the mean time taken to start and disappear the scatter of the *Neikuri*. The *Neikuri* was significantly associated with *Naadi* when a drop of oil (25 – 50µL) was placed over the surface of the urine. When the volume of the oil drop increased the time taken to start and disappear the scatter of the *Neikuri* increased. The spreading was decreased when used 10µL and 20µL of oil drop and significant variation also observed with *Naadi*.

Table 5: Mean time taken to start and disappears the scatter of the *Neikuri* with volume of oil drop

Amount of oil used (μL)	Mean time taken to start scatter (seconds)	Mean time taken to disappear scatter (seconds)
10	468.7 \pm 198.7	1547.9 \pm 681.1
20	338.4 \pm 205.4	1134.8 \pm 201.7
25	153.1 \pm 149.7	912.1 \pm 289.3
50	209.6 \pm 161.8	969.3 \pm 122.3
100	345.9 \pm 176.4	1247.7 \pm 659.8

Height of the placement of oil from the urine surface

Less than 2mm distance was observed as suitable to place the oil from the urine surface. Formation of wave observed when place the oil above the height of 2mm.

Status of the utensil

The *Neikuri* was significantly related with *Naadi* when the utensil opened, even though no association observed when test the *Neikuri* in close chamber. Further minimum spread was noticed and prolonged time taken to start scatter (857.6 \pm 356.5 seconds) and disappear the scatter (1897.2 \pm 547.4 seconds) of the *Neikuri* in closed chamber. In addition scatter was not observed in some attempts, when test the *Neikuri* in closed utensil.

DISCUSSION

In the current study we analysed the procedures of testing the *Neikuri*. A number of previous studies documented the procedures of testing the *Taila bindu pariksha* and *Neikuri*,^[3,4,6] even though elaborate studies have not been documented to the best of our knowledge. In the current study, we thoroughly studied the methods of testing *Neikuri*. Twelve parameters as variety of the oil, collection time of the urine, time duration between the collection of urine and test performed, time duration for examine the *Neikuri*, material, colour, size and shape of the utensil that used to test, volume of urine with size of the utensil, volume of oil drop, height of the placement of oil from the urine surface and status of the utensil have studied in patients to standardize the *Neikuri*. Five variety of sesame oils were studied in the current study. The correlation was not considered between *Neikuri* and *Naadi* when testing *Neikuri* in previous studies. Specially in the current study the correlation was considered between the reading of *Neikuri* and *Naadi* of the particular subjects and according to that correlation the procedures were standardized, where it applicable.

One previous study on *Neikuri* towards standardisation reported and the study conducted by using healthy subjects.^[6] A study on *Taila bindu pariksha* has tested the parameters such as material, shape and size of the utensil, volume of the urine, size

of the oil drop, height of the placement of oil drop from the surface of the urine and variety of the oil, to standardize the procedures using healthy volunteers.^[3] Another study reported^[4], the time duration between the collection of urine and the test performed, in addition to the parameters studied in the above study.

The current study confirmed that, the *Neikuri* using the sesame oil prepared from black and mixed seeds has significant association with *Naadi*. However the time difference was observed in these two oils, from commencement to disappear the scatter of the *Neikuri*. The time to perform *Neikuri* in black and mixed sesame seeds oil were 912.1 \pm 389.3 seconds (15.2 \pm 6.5 minutes) and 1228.2 \pm 582.9 seconds (20.5 \pm 9.7minutes) respectively. When compare these two oils, the oil derived from black sesame seed is appropriate for testing *Neikuri*. Previous studies confirmed that the oil derived from black sesame seed is suitable for *Taila bindu pariksha*.^[3,4]

A significant association was observed with *Neikuri* and *Naadi* when the *Neikuri* examined from sun rise to before sunset, whereas there was no significant association observed, when *Neikuri* tested in night. These finding indicted that the rays of sunlight may influence in the reading of *Neikuri*. In addition the *Neikuri* was not relevant with *Naadi* when the utensil was closed. It indicated the environmental pressure or the rays of sunlight may influence in *Neikuri*. Experience gained that, *Neikuri* could readable clearly and easily under the sunlight than the artificial light sources. The sage Theraiyar stated that, early morning urine good to examine the *Neikuri*. Further the sage has explain that the rules not need to follow when examine the *Neikuri* to a patient.^[1] In addition the sage Agasthiyar explains that the *Neikuri* need to examine in sunlight.^[2] In addition there was no significant variation observed in *Neikuri* when collect the urine before and after the breakfast.

Significant association noted with *Neikuri* and *Naadi* when testing the *Neikuri* within one and a half an hour from the collection of the urine. A reported

finding indicated the test need to perform within two hours^[4] and this finding testify the current study and the literature, *Noi nadal Noi muthal naadal thirattu*^[1] also documents that the *Neikuri* need to test before one and a half an hour (one *Moohoortham*- 90 minutes) from collection of urine. *Neikuri* doesn't associate with *Naadi* when examine the *Neikuri*, after one and a half an hour from the collection of the urine. It may due to the influence of bacterial activities^[4] or reduction of temperature in the collected urine.

There was no influence observed in the colour of the utensil when testing *Neikuri*. However difficulty was experienced on getting reading when testing different colours of urine (transparent to dark colour) in colour utensil with unaided eye. Results indicated that easy to get result when using colourless glass and clay utensil. However the *Neikuri* was not significantly associated with *Naadi* when test the *Neikuri* in a clay pot. Low and high time was taken to spread, start to scatter and disappear the scatter of the *Neikuri* when the clay pot in cool and hot environment respectively. This shows the influence of the temperature (cool/hot) of the clay utensil was interfered with *Neikuri*. The clear shape and spreading of *Neikuri* was observed in petri dish when compare with glass bowl. Pressure may influence in reading of *Neikuri* and it is possible to maintain similar pressure in all directions when use petri dish. One of the ancient text book *Theraiyar arulichcheitha Neernirakkuri Neikurich saththirangalin moolamum* documents that the *Aadi kalasam* (glass utensil) suitable for testing the *Neikuri*^[1] and previous studies^[3,4] used glass utensil to test the *Neikuri* as well. The literature *Kannusaami pillai* documents glass utensil and clay pot may use to test the *Neikuri*.^[2]

Variations were observed in time taken to start and disappear the scatter of the *Neikuri*, among the different size of the utensils used. Although it was not affected the correlation between reading of *Neikuri* and *Naadi*. The minimum time taken to start and disappear the scatter of *Neikuri* was observed in 3.5inch (8.9cm) of petri dish with 50ml of urine. In general, our study indicated that the *Neikuri* not depended neither in the amount of the urine nor the size of the utensil used. Small diameter of utensil could be used to test *Neikuri* in the condition of availability of low volume of urine. Although, contradicts noted with reported studies. A previous study conducted using 8 inch (20cm) diameter of petri dish and 200ml of urine^[4] and another research reported that 3.88 inch (9.8 cm) diameter glass utensil with 30ml of urine as standard.^[3]

The shape of the utensil does not affect the *Neikuri* and shape of spreading. However the circular shape of utensil suitable to identify the fully shape of the spreading of the *Neikuri* and it was easy to get reading of the *Neikuri*. Similar finding observed in reported studies.^[3,4]

The volume of oil drop was interfered with *Neikuri*. There was significant association with *Naadi* and a minimum time variation observed in *Neikuri* when using 25-50 μ l volume of oil drop. A discrepancy noted with previous studies,^[3,4,6] indicated 12 μ l oil drop as standard to perform *Neikuri*. A minimum spread was observed in *Neikuri* when the volume of oil drops less than 25 μ l. This may due to the restriction of reaction between oil and urine. The time taken to start and disappear the scatter of *Neikuri* was increased when using the volume of oil drop above 50 μ l. In addition a toothpick may also use to take an oil drop instead of using micropipette.

Our study confirmed the height to place the oil drop from the urine surface was less than 2 mm. The current finding contradicted with previous studies. Two studies^[3,4] indicated that, 1cm as standard height to place the oil drop from the surface of urine. Formation of waves observed on the urine surface if the height increase than 2mm and it may affects the spreading and formation of the shape of the *Neikuri* by foaming waves.

There is a possibility to affect the spreading and shape of the *Neikuri* by wind.^[1] A room with natural sun light advisable to test the *Neikuri* (need to confirm to switch off the fans). Clean and dry utensil need to use to test *Neikuri*. False results was observed when examine the *Neikuri* without washing the utensil. It is preferable to use clean separate vessels to collect the urine from the subjects and transfer the urine into the test utensil when conducting the test. These procedures could facilitate to avoid false results. Keep the glass utensil on a white marble facilitate the observation and reading the *Neikuri*.

The patients below 10 years did not include in the study. Because the literature *Noi nadal Noi muthal naadal thirattu* documents, that the *Naadi* could not correctly readable in *Paalar* (aged below 10 years).^[1]

There may a number of factors including surface tension of urine and oil, molecular weight, shape of the molecules and polarity of the molecules in the urine and oil, contents of the urine, environmental pressure and rays of sun light may influence in *Neikuri* and formation of the shape on spreading. Further details studies are warranted to identify the mechanism of *Neikuri*.

CONCLUSION

We conclude the standard procedures to test the *Neikuri* as, circular glass utensil (3.5 inch petri dish preferable) and 25-50µl drop of sesame oil derived from black sesame seeds need to use. The test needs to perform with in one and a half an hour from the collection of urine and the oil need to place less than 2mm height from the surface of the urine. Further the time taken to test *Neikuri* ranging from 8.7 to 22 minutes under sun light. These procedures may consider as standard protocol for testing *Neikuri*. Further studies will be needed for confirmation.

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