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Photocolorimeter – Can it be a novel application to quantify Avila Mutrata?

Research Article

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Abstract

Prameha has been described as one of the *Mahagadas* and *Anushangi vyadhi*. It is also a disease which is associated with derangement in *Ojus* (~Immunity). The disease is manifested as *Prabhootavilamootrata* (Polyuria with turbidity in urine). *Madhumeha* can be closely correlated to Diabetes mellitus. Glycosuria is a striking feature of the disease, which alerts the patient. It happens when the glomerulus filters more glucose than the proximal tubule can reabsorb. It can be due to elevated plasma glucose or renal glucose absorption impairment, or both. Excessive *Kleda* in the condition of *Madhumeha* gets mixed with other doshas and dhatus and forms *Vikrita avastha*. *Kleda* has two routes of expulsion from the body viz; *Sweda* and *Mutra. This can lead to the Avilata mutrata in Madhumeha. Avilata* is a condition related to light absorption, diffusion and Chroma/Chrome. liquid. Hence *avilata* is related with transparency of an object and its optical density (OD). Therefore, COLORIMETER instrument was chosen for the mutra *pariksha*, which works on the principles of OD. Colorimetric analysis can become a simple, OPD based, cost effective, and specific quantitative analysis of Urine which can be used to translate the principles of Ayurveda into practice and for better management and monitoring of *Madhumeha*.

Key Words: *Madhumeha, Avila mutrata, Kleda, Photo Colorimeter, Mutra Pariksha.*

Introduction

Prameha has been described as one of the *Mahagadas* and *Anushangi vyadhi*. It is also a disease which is associated with derangement in *Ojus* (~Immunity) (1). *Acarya Caraka* states that all those things that produce excessive *Kapha*, *Meda*, *Mūtra*[2] are causative factors of Prameha. All the *ahara-vihara* (Diet and lifestyle factors) having *snigdha* (unctuous), *sita* (cold), *guru* (heavy), *picchila* (slimy), *madhura* (sweet), *slakshna* (smooth) properties, those which increase kapha (2) and vitiate *dushya* are the causative factors of *Madhumeha.* These factors mainly cause excessive burden on metabolism at cellular level resulting in intermediate metabolites. The compromised metabolism leads to excess production of *meda, kleda lasika*, *mutra*, *sveda* and deposition of *medha* at various sites.

The disease is manifested as *Prabhoota avilamootrata* (3) (Polyuria with turbidity in urine). These are striking features which draw the attention of patient as well as clinician to suspect the disease. Ayurveda has also explained certain *Purvarupa* (premonitory symptoms) which are actually long lasting and of high prognostic predictive value.

The diseases afflicting urine are classified into two types *Mutra Apravrittaja Rogas* i.e. diseases leading to less quantity of urine and *Mutratipravrittaja Rogas* i.e. diseases leading to excess quantity of urine, *Prameha* being *Mutratipravrittaja roga. Prameha* as the word says, *"prakarshena prabhutam prachuram varam varam va mehati"* (4). It is further subdivided into 20, based on physical abnormalities of urine. However, all *Prameha* if neglected may lead to *Madhumeha* in long term (5). *Madhumeha* has been extensively described in ancient *ayurvedic* texts of all times including *brihadtrayi and laghutrayi. Acharya Charaka* is very specific while describing the types of *Prameha* on the basis of onset as *Sahaja* / *Jatah Prameha* and *Apathyanimittaja prameha*, which can be correlated to Type 1 and Type 2 Diabetes respectively.

By consumption of *kaphakara nidana,kapha dosha* gets aggravated and gets *prasrita* all over the body. As body is already in a *shithila* state, *kapha* gets conflated with *meda,mamsa* and other *dhatus* with more *kleda bhava. Dhatus* with *kleda bhava* reaches *basti* and patient passes the urine with more frequency and quantity. *Basti or mootrashaya* is the main site of pathogenesis (6).

Prameha has been described as anushangi (7) which means it is long lasting. Therefore, all efforts should be made to prevent and control it. *Madhumeha* passes through 3 stages of severity based on

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involvement of *dosha* and *dhatus*. Accordingly, prognosis has been described. *Prameha* is a general term which describes all 20 varieties of urinary abnormalities with excessive urine output. *Madhumeha* is *Vatika* variety of *Prameha* which is a chronic, fatal and incurable state. Another variety of *Madhumeha* is due to *Maragavarana,* which is not as fatal as *Vatika* variety.

Kaphajameha usually having good prognosis, on association with all *poorvaroopas,* attains bad prognosis. Similarly, *Pittaja Prameha* usually considered as *Yapya* attains bad prognosis (*Pratyakhyeya*) when associated with all poorvaroopas (8).

The severity of *Asadhyata* increases when associated with *poorvaroopas. Vatajapramehas* have already been described as *Asadhya* (8) but this term has to be analytically interpreted among the two clinical types of *Vatajamehas* i.e. Dhatu *KshayaJanya* and *MargavaranaJanya*, as *Margavarna janya Madhumeha* is *Krichra sadhya* (9).

Madhumeha can be closely correlated to Diabetes mellitus (DM). DM is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves (10). Glycosuria is a striking feature of the disease, which alerts the patient. It happens when the glomerulus filters more glucose than the proximal tubule can reabsorb. It can be due to elevated plasma glucose or renal glucose absorption impairment, or both. The term "diabetes" was first coined by Araetus of Cappodocia (81-133AD) which means to siphon. Later, the word mellitus (honey sweet) was added by Thomas Willis in 1675 after rediscovering the sweetness of urine and blood of patients (first noticed by the ancient Indians). It was only in 1776 that Dobson firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness. An important milestone in the history of diabetes is the establishment of the role of the liver in glycogenesis, and the concept that diabetes is due to excess glucose production by Claude Bernard in 1857 (11). Indian scientific literatures identified the disease as *madhumeha / kshaudrameha* noting that the urine would attract ants and look like honey.

The International Diabetes Federation has estimated that globally there are 415 million people with diabetes in 2015 and is predicted to increase to 642 million by 2040. In the world, India stands second with 69.2 million people with diabetes and another 36.5 million with prediabetes which is a high-risk condition for diabetes and cardio-vascular disease (12). The Cost of Diabetes in India (CODI) study was a large community-based survey of diabetes costs. According to the results of the CODI study, ambulatory care constitutes 65% cost whereas hospitalization cost is 35%. Therapy cost is 31% of which specific antidiabetic drug cost is only 17%. Ambulatory care including monitoring and doctor visits constitute 34% costs (13). Hence there is a need for more cost-effective evaluation measures and treatment measures which could reduce the economic burden on the nation.

Concept of *Kleda* (14,15,16)

One of the crucial and principal components of the human body that is least discussed and explored is Kleda. The term Kleda can be inferred as hydration/ moisture. Kleda plays an essential role in maintaining the physiology and manifestation of disease when in imbalance. It is the fundamental constituent in the pathogenesis of diseases associated with Pitta and Kapha Dosha. The entire activity of fluids, hydration, and fluid balance comes under the broad umbrella of the concept of Kleda. Kleda is the physiological requirement of the body as mentioned in the context of Ahara Parinamakara Bhava (Food Bio transforming factors). Here Kleda imparts Shaithilyam (Softness without integration) to the taken Ahara i.e disintegrating complex food particles, it is nothing but the moisture, which induces wetness to food. Prakrita Kleda (Normal) can turn into Vikrita Bahu Kleda (Abnormally surged). The condition of Bahu Kleda is referred to as Klinnata. This *kleda* has to routes to get expelled from the body viz; *Sweda* and *Mutra.*

In the chapter of *Prameha* special importance for mutra and mutra *pariksha* has been given in order to diagnose, treat and determine the curative stage of the disease. Therefore, the concept of mutra *pariksha* with respect to *avilata* is of much importance.

Avilata is one of the *pratyatma lakshanas* told for the *Pramehi* mutra in all the classical texts of Ayurveda. *Avilata* has been explained in various contexts of Ayurveda. Upon detailed study of all the references, understanding of *avilata* is summarised under, "*samala", "ishat picchila", "Dhusara*". They all mean something that is "Not clear", "Entity with sediments", "Non-Transparent" or "Translucent", "Near-opaque". In Ayurveda Shastra, the terms *avilata* has been used for body components like *Netra* (17,18,19), *Mutra, Rakta*, which all are either Transparent/Liquid in nature, conveying that *Avilata* is a condition related to light absorption, diffusion and Chroma/Chrom. liquid. Hence *avilata* is related with transparency of an object and its optical density (OD). Therefore, COLORIMETER instrument was chosen for the mutra *pariksha*, which works on the principles of OD.

Colorimeter is an instrument used for the quantitative measurement of coloured substance in a solution. The instrument is operative in the visible range of the Electromagnetic spectrum. It is the most common analytical technique used in biochemical estimation in clinical laboratory. It involves the quantitative estimation of colour. A substance to be analysed calorimetrically should be coloured or it should be capable of forming chromogens (Coloured complexes) through the addition of reagents.

Principle of Colorimeter

The colorimeter works on two principles pertaining to absorption of light and solution chemistry.

When a monochromatic light passes through a coloured solution, some specific wavelengths of light are absorbed which is related to colour intensity. A light ray of a certain wavelength, which is specific for the assay is in the direction of the test solution. The light passes through a series of different lenses and filters. The coloured light navigates with the help of lenses, and the filter helps to split a beam of light into different wavelengths allowing only the required wavelength to pass through it and reach the cuvette of the standard test solution. When the beam of light reaches cuvette, it is transmitted, reflected, and absorbed by the solution. The transmitted ray falls on the photodetector system where it measures the intensity of transmitted light. It converts it into the electrical signals and sends it to the galvanometer. The electrical signals measured by the galvanometer are displayed in the digital form **(Figure 1).** Formula to determine substance concentration in the test solution is by calculating the relation between absorbance A, concentration c (expressed in mol dm-3) and path length t (expressed in cm), as given by Beer-Lambert's law. The amount of light absorbed or transmitted by a colour solution is in accordance with two laws; viz. Beer's and Lambert's law. Beer's law states that the concentration of the substance is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the transmitted light. Lambert's law stares that when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the light path through the material increases. Equation: $A = \in cl$, where $\in$ is the molar extinction coefficient, c is the concentration, t is the path length and is constant for a given substance at a given wavelength (20).

The present investigations in medical practice for diagnosing, managing and monitoring the disease *Prameha* are mostly invasive and are based on the principles and knowledge of Diabetes Mellitus and evaluation related to it. Ayurveda rightly emphasis the Urine examination as the foremost test in cases of *Madhumeha.* Urine examination is non-invasive and a relatively uncomplicated procedure. But the mostly used, present techniques for Urine examination pertaining to estimation of sugar is subject to high extent of variability. The dip sticks test is based on the change in colour of the strips when dipped into the urine sample. The results can become highly subjective as perception and interpretation of colour is highly variable from one individual to other. The standard colours assigned for the presence of particular quantity of urine sugar are also diverse and non-identical for different company products. Hence there is a need for simple, handy, OPD based, non-invasive, cost effective, and specific quantitative analysis of Urine in order to translate the principles of Ayurveda into practice and for better management and monitoring of Madhumeha. Thus, Colorimetric analysis was chosen for the assessment of Mutra and values were calculated and tabulated for normal and affected urine samples.

Methodology invovlved

Product specification-

- Product code St-211 Std.
- Glass Filter 8 No.
- Mini volume 1ml.
- Display 2 ½ digit LED
- Range 400-700 nm
- Output OD (0 to 1.99).
- Resolution OD: 0.01
- Accuracy 0.5%
- FSI Detector photocell photoiode
- Lightsource 6.8v, 300mA.

Colorimetric assessment of urine

Calibration of the device

- At 1st the digital photo colorimeter was checked for all the normally functioning parts.
- Instrument was connected to power source and filter was set for RED – 680 nm as the solution here being used was Benedict's reagent which is blue in colour.
- Initially cuvette filled with distilled water was kept in the chamber and reading was adjusted to zero **(Figure 3).**
- This procedure was done three time to attain accuracy.

Preparation of standard solutions

- Standard solutions were prepared for 2.5%, 5%, 10%, 15% sugar concentration.
- For instance, 2.5gm of sugar was dissolved in 100 ml of distilled water, stirred well to get 2.5% glucose standard solution **(Figure 2).**

Procedure of assessment

- Cuvette was filled with Benedict's reagent, and kept in chamber. Value was recorded.
- Equal amount of Benedict's reagent and standard solution were mixed and taken in 4 different (2.5%, 5%, 10%, 15%) test tubes.
- They were kept in thermal bath for 2mins and later kept for cooling for another 5 minutes.
- Colorimetric value for all the standard solutions were marked and plotted in a graph **(Figure 4).**
- Later each time while assessing the colorimetric value of the urine sample similar procedure was followed and checked in the plotted graph for the concentration of sugar in the unknown urine sample.

Observations and Results

Standard solutions attained Deep Blue, Green, Orange and Brick red for 2.5%, 5%, 10%, 15% concentrations respectively, at the end of 5 mins of cooling (Figure 2). Colorimeter showed "0" for distilled water and highest value of "1.89" for Benedict's reagent, signifying that maximum amount of light was transmitted without being absorbed as the sample didn't not contain any solvent which would react with Benedict's reagent.

The current method used of Colorimetric assessment of urine is superior and augmented to the regular Benedict's test (BT) for estimation of sugar. The urine sugar scale used in BT is very broad with a range of 500mg/dl per grade based on conversion of urine colour. By using the photo colorimeter method, a more precise and narrower urine sugar value can be estimated based on the extent of absorption of light.

Urine sample collected from the patients can be of various colours ranging from colourless and red/ brown. Hence all the samples were uniformly treated with Benedict's reagent, so that a light beam of single uniform wavelength could be passed through the solution.

If wavelengths of light from a certain region of the spectrum are absorbed by a material, then the materials will appear to be of the complementary colour. Filter in the colorimeter is used to select the colour of light which the solute absorbs the most, in order to maximize the accuracy of the experiment, hence the colour of the absorbed light is the 'opposite' of the colour of the specimen. In the present study as the Benedict's reagent is a blue coloured solution, a light beam of complementary colour i.e red was used for analysis of the samples, as it enhances the accuracy of the experiment.

Similarly normal urine samples too showed values "1.77-1.85" indicating nearly no absorption of red light due to absence of Sugar. But standard solutions of 2.5%, 5%, 10%, 15%, 20% showed values of 1.7. 1.65, 1.3, 1.2 and 1 respectively (**Table 1**), indicating least transmission and maximum absorbance of light in 20% standard solution due to maximum concentration of sugar in urine. These values were plotted against the graph and every time an unknown urine sample was analysed, the colorimetric galvanometer reading was plotted in graph and concentration was determined. Thereby the change in colour, transparency and thickness of the solution during the course of analysis can be related to *Avilata* and its assessment.

Table 1: Colorimetric reading of Anavila mutra of apparently normal individual and Standard solutions

Category	Colorimetric Value
Plain water (Std)	0.0
Benedict's reagent	1.89
Anavila mutra (Apparently healthy individual)	1.77
2.5% Std solution (2.5g/dl)	1.70
5% Std solution (5g/dl)	1.65
10% Std solution (10g/dl)	1.30
15% Std solution (15g/dl)	1.2
20% Std solution (20g/dl)	1

Conclusion

The chapter of *Prameha* special importance for mutra and mutra *pariksha* has been given in order to diagnose, treat and determine the curative stage of the disease. Therefore, the concept of mutra *pariksha* with respect to *avilata* is of much importance. *Avilata* is related to transparency of an object and its optical density (OD). Therefore, COLORIMETER instrument was chosen for the mutra *pariksha*, which works on the principles of OD and on Beer-Lambert's law. standard solutions of 2.5%, 5%, 10%, 15%, 20% showed values of 1.7. 1.65, 1.3, 1.2 and 1 respectively, indicating least transmission and maximum absorbance of light in 20% standard solution due to maximum concentration of sugar in urine.

Limitation of the study was that the Photo colorimeter that was used, was a basic model without much of high-end analysis as it had only sensor and data processors without a computer device. Similar study can be conducted in a varied range of standard solutions through Spectrophotometer for better and more precise results. Hence, Colorimetric analysis is a simple, handy, OPD based, non-invasive, cost effective, and specific quantitative analysis of Urine which can be used to translate the principles of Ayurveda into practice and for better management and monitoring of *Madhumeha*.

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Figure 1: Principle of Photo Colorimeter

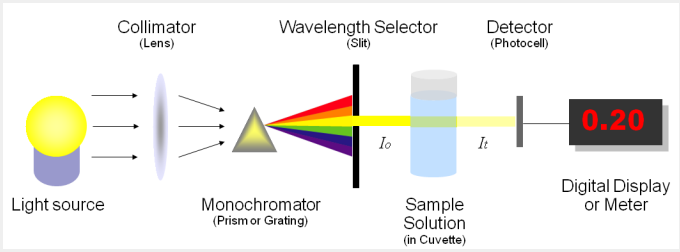


Figure 2: Standard solutions

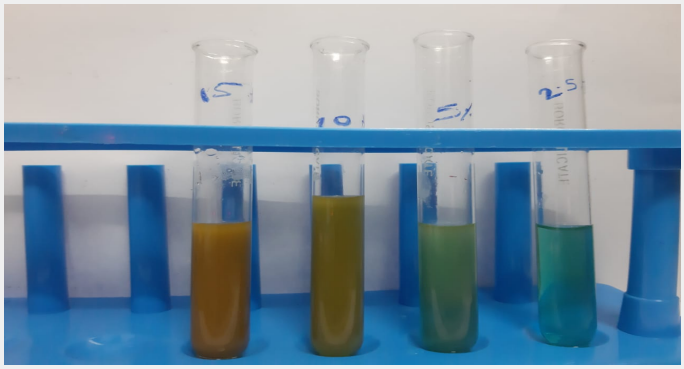
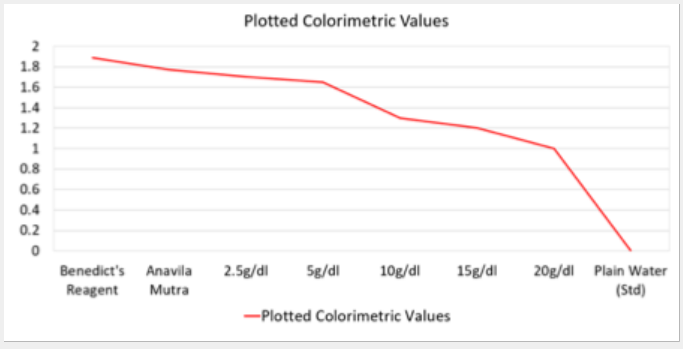


Figure 3: Colorimetry reading for distilled water and Benedict's reagent

Figure 4: Plotted colorimetry values



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