Analysis of biomarkers in sweat and its comparative study with Blood

I.Jeya Daisy, B.Vinoth Kumar

Abstract: ***In the current medical field, the many methods used to analyze the bio fluids for biomarkers are blood, urine, saliva and sweat. Blood testing by sampling is to be done frequently for analysis from patients rendering them prone to higher chances of infection and storage for the procedure also proves difficult. Investigations into biomarkers contained in Sweat have so far been limited. Compare to blood, most of the drugs are accumulated in sweat. It is noteworthy that certain biomarkers like nitrogenous compounds, metal and non-metal ions, metabolites and xenobiotics are found in sweat in such the amount approximately equal to other biofluids. In this paper, the sweat collection and its analysis for determining biomarkers are proposed. Additionally, comparison of biomarkers in blood and sweat had done and tried to prove that sweat is noninvasive, cost effective and accurate analysis compared to blood.***

Keywords: biomarker; spectrophotometer,atomic emission spectrophotometer,heavy metals

I. INTRODUCTION

In sweat glands, cutaneous attachments similar to hair follicles are exocrine glands (possess ducts). They are present all over the surface of the body on the epidermal layers. Glands can be characterized by secretion modes or morphology. Sebaceous glands are classified holocrine and eccrine. This classification of sweat glands into eccrine and apocrine was introduced by Schiefferdecke. Many of the literature works on eccrine or apocrine sweat glands. Apart from these two is a third type of sweat gland called the apoeccrine which fall under neither eccrine nor apocrine discovered in 1987. Although recent studies confirm their existence, their development and their role in sweating is yet to be understood fully. It shows a significant structural and functional difference and much complication from the two by opening into the hair follicles or freely onto epidermal surfaces. Literatures also refer to the eccrine as the small sweat gland and the apocrine as the big or scent gland. The apoeccrine gland might also have been called as the mixed type sweat gland.

II. BIOLOGY OF ECCRINE SWEAT GLAND

The merocrine type of sweat glands is widely existing in the body. It produces an apparent odorless form of sweat composes water. The sweat produces white deposit with high salt concentration when evaporated The glands are found throughout the stretch of the skin which highly concentrated in palms, soles of feet and in the forehead areas. The eccrine sweat also contains glycoprotein's, sugars, electrolytes and amino acids.

The most and majority constitute of eccrine sweat which is collected from the forehead is nitrogenous compounds like lactic acid, amino acid, electrolytes and urea.

III. DETERMINATION AND SAMPLE PREPARATION FOR DETECTION OF BIOMARKERS IN SWEAT

A. Amino Acid

In sweat, amino acid is detected by Ninhydrin reaction (2,2-dihydroxyindane-1,3-dione). Ninhydrin is used to predict ammonia and/or primary and secondary amines in the sweat. The free amines on reaction with Ninhydrin yield a deep blue or purple color called as Ruhemann's purple, while amino acids hydroxyproline and proline gives a yellow complex. Ninhydrin also gives a positive reaction with peptides and peptones.

Sample preparation for detection of Amino Acid

The 80ml of Ninhydrin is mixed with 1ml of sweat with the room temperature of 25ºC.

B. Lactic acid

Lactic acid reacts photo chemically with Fe (III) and reduces it to Fe (II) when irradiated with UV light. The amount of Fe (II) produced determines the amount of lactic acid content in the sample which found electrochemically using spectrophotometry.

Sample preparation for detection of Amino Acid

The 1ml of sample is fusioned with 86.5ml of distilled water and 0.04g of FeCl3with the room temperature of 25ºC.

C. Sodium

The amount of sodium content in sweat about 500 mg sodium/lb sweat (and ranges from 220 to 1,100 mg).Sodium losses resulting imbalance in body fluids. so there is a need to monitor sodium contents in sweat.

Sample preparation for detection of Sodium

Carefully open an oral rehydration sachet and empty the contents into a clean 250 ml beaker. Add about 150 ml distilled water and gently swirl the contents until dissolved. Transfer the solution into a 200 ml volumetric flask and wash the beaker with small amounts of distilled water. Finally, make up the flask to exactly 200 ml and mix thoroughly. Formulate a 1/50 dilution of the redissolved sachet solution by accurately pipette 2 ml of the solution into a 100 ml volumetric flask and making up to 100 ml with distilled water

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D. Potassium

Along with sodium, potassium is one of your body's most important electrolytes, which are minerals in bodily fluids that contain an electric charge. Body's cells use electrolytes to carry electrical impulses throughout the body. These charges help cells to communicate with each other and give the details about taste, see, smell, touch, and hear. Nearly 70% of the potassium is found in sweat.

Sample preparation for detection potassium

1. Stock Solution: Dissolve 0.1907 g of KCI (analytical reagent grade), dried at 110 deg. C, in deionized distilled water and make up to 1 liter. 1 ml = 0.10 mg K(1OO mg/l).

2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

E. Heavy Metals

Heavy metals are present only in smoking person sweat and person who working in toxic atmosphere like mining industry. Here, analysis is done on smoking person's sweat.

Heavy metals determined are Zinc, Lead and Cadmium.

Sample preparation for detection of Heavy metal

Lead

Dissolve 1.000g.of lead metal in 50ml. of 2M nitric acid. Dilute to 1 liter in a volumetric flask with de-ionised water. Dissolve1.5980g. of lead nitrate (Pb(NO3)2) in 100ml. of de-ionised water. Dilute to 1 liter in a volumetric flask with de-ionised water.

Zinc

Dissolve 1.000g. of zinc metal in 30ml. of 5M hydrochloric acid. Dilute to 1 liter in a volumetric flask with de-ionized water. Dissolve 1.2450g. of zinc oxide (ZnO) in 5ml of de-ionised water followed by 25ml. of 5M hydrochloric acid. Dilute to 1 liter in a volumetric flask with de-ionised water

Cadmium

Dissolve 1.000

g. of cadmium metal in 20ml. of 5M.hydrochloric acid and 2 drops of conc. nitric acid. Dilute to 1litre with de-ionised water. Dissolve 2.0360g. of cadmium chloride in 250 ml de-onised water. Dilute to 1 liter in a volumetric flask. Dissolve 2.1032g. of cadmium nitrate in 250ml.of de-ionised water.

IV. ANALTICAL METHODS USED TO DETECT BIOMARKERS IN SWEAT

A. UV-Spectrophotometry

A PC-based Double Beam UV-Spectrometer2206 was used for detecting amino acids, lactic acid and proteins.

Reasons for Choice

- 1 nm Bandwidth
- Base line calibration and Automatic source Optimization
- Complies with International Pharmacopoeia
- It is PC-based
- Single & Multi Wavelength
- Multi Scan facility and has Time/Kinetic Scan operating modes.

B. Flame emission spectrophotometry

This is a type of inorganic analysis which determines the presence of the element and also the quantity by the intensity of the emitted flame from the flame used in the process. Moreover, sample preparation is not required for this method.

C. Atomic Absorption Spectrophotometry

This technique uses the absorption of optical radiation emitted by the free atoms of the elements while in the gaseous state.

V. ANALYSIS OF BIOMARKERS AND ITS CONCENTRATION

A. Amino Acid (phenylalanine)Analysis and Results

Amino acid and Lactic acid were analyzed by using double –beam uv-spectrophotmeter. 10 different persons sweats were collected and samples were prepared to detect amino acid.

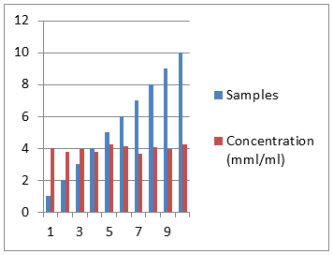

Fig 5.1 Amino Acid Concentration in the samples

In fig 5.1 shows that the amino acid (phenylalanine) in sweat was varied widely in between 3.68 to 4.28(mml/1ml).

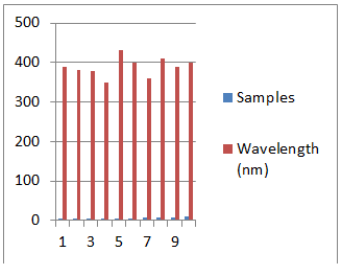

Fig 5.2 Wavelength detection of Amino Acid

In fig 5.2 shows that the wavelength detection of Amino acid (phenylalanine) in sweat were in between 375 to 420nm.

B. Lactic Acid Concentration in the samples

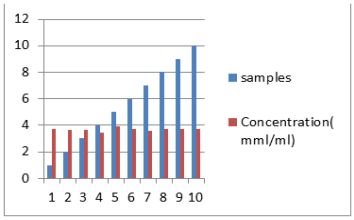

Fig 5.3 Lactic Acid Concentration in the samples.

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In fig 5.3 shows that the Lactic acid in sweat was varied widely in between 3.70 to 3.91(mml/1ml).

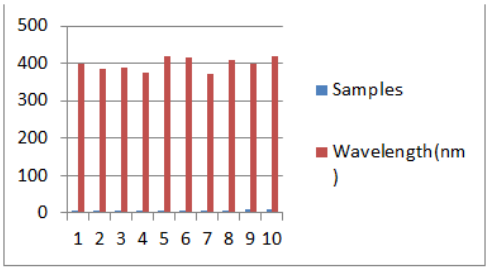

Fig5.4 Wavelength detection of Lactic Acid

In fig 5.4 shows that the wavelength detection of Amino acid (phenylalanine) in sweat was between 3605 to 430nm.

D. Sodium Analysis and Results

Several researchers approve that the ion content of sweat falls within or close ion content of blood. Normally sweat contains 3381 ± 253 ppm of sodium. Sodium was analyzed by Flame Emission spectrophotometer.

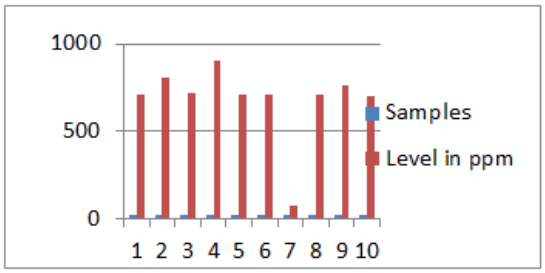

Fig 5.5 Sodium Concentration in the samples

In fig 5.5 shows that the Sodium concentration for 10 different samples were varied widely in between 706.66 to 900.21 ppm.

D. Potassium Analysis and Results

Normally sweat contains 195.5 ± 39.1 ppm of potassium. of sodium. Sodium was analyzed by Flame Emission spectrophotometer.

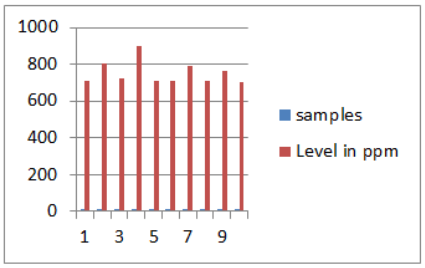

Fig 5.6 Potassium Concentration in the samples

Potassium concentration for 10 different samples were varied widely in between 706.22 to 802.11 ppm showed in Fig 5.6.

E. Heavy Metals Analysis and Results

Only Smoking persons sweat contains heavy metals. Detection of this heavy metal in sweat is used to give an exposure about the person's body disorders. An important excretory pathway for zinc and copper is through sweat. A level of zinc was lower in sweat from females because of menstrual and other losses. The arm-bag collection method is used to collect the sweat.

Amount Of Sweat Collected- 5ml
Collected Date: March-15-2017
Equipment Used: Atomic Absorption Spectrophtometer
Standard: Lead
Sample

Element	0.2 ppm	0.4 ppm	0.6 ppm	0.8 ppm	1.0 ppm
Lead	0.316	0.335	0.341	0.344	0.353
	0.320	0.334	0.340	0.345	0.351
	0.356	0.334	0.339	0.345	0.351
Mean	0.356	0.334	0.340	0.345	0.352
SD	0.0006	0.0004	0.0013	0.0004	0.0009
RSD %	0.2	0.1	0.4	0.1	0.3

Table 5.7 Lead Standard

Sample

Values mg/L			Mean	SD	RSD%
Replicate 1	Replicate 2	Replicate 3			
0.002	0.007	0.005	0.005	0.0024	51.5

Table 5.8 Lead Constitutions InSweatstandard: Cadmium

Element	0.2ppm	0.4ppm	0.6ppm	0.8ppm	1.0ppm
Cadmium	0.321	0.404	0.459	0.541	0.602
	0.322	0.404	0.458	0.521	0.603
	0.321	0.403	0.460	0.538	0.603
Mean	0.322	0.404	0.459	0.533	0.603
SD	0.0005	0.0005	0.0006	0.0106	0.0007
RSD %	0.1	0.1	0.1	2.0	0.1

Table 5.9 Cadmium Standard

Sample

Values mg/L			Mean	SD	RSD%
Replicate 1	Replicate 2	Replicate 3			
0.135	0.135	0.135	0.135	0.0003	0.2

Table 5.10 Cadmium constitutions in sweat

Standard: Zinc

Element	0.2ppm	0.4ppm	0.6ppm	0.8ppm	1.0ppm
Zinc	0.240	0.297	0.340	0.344	0.413
	0.242	0.200	0.336	0.337	0.410
	0.244	0.298	0.337	0.345	0.408
Mean	**0.242**	**0.298**	**0.338**	**0.342**	**0.410**
SD	0.0012	0.0015	0.0017	0.0021	0.0025
RSD %	5.3	1.6	0.5	1.2	0.6

Table 5.11 Zinc Standard

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Sample

Values mg/L			Mean	SD	RSD%
Replicate 1	Replicate 2	Replicate 3			
0.002	0.001	0.001	0.002	0.0009	56.2

Table 5.12 Cadmium constitutions in sweat

VI. SWEAT RESULTS

By measuring the above biomarkers, the abnormalities were detected from the sweat and the smoking person sweat contains the high level of cadmium toxic metal, Hence, the person was advised that smoking should be avoided. To conclude, persons who have above or below the normal level mentioned above in the table 6.1, corresponding abnormalities can be detected with help of sweat analysis.

Biomarkers	Normal Level	Measured Level	Normal/ Abnormal
SODIUM	3128 ppm – 3358 ppm(BLOOD)	706.66 (SWEAT)	**Normal**
POTASSIUM	136.85 ppm – 195.5 ppm(BLOOD)	179.16 (SWEAT)	**Normal**
AMINO ACID	110-360(umol/ml)	4mml/1ml	**Abnormal**
LACTIC ACID	0.5 to 1mmol/l	0.37 mmol/l	**Normal**
LEAD	0-1.5ug/dl	0.5ug/dl	**Normal**
CADMIUM	0.5ug/l	13.5ug/dl	**Abnormal**
ZINC	0.66 - 1.10mcg/ml	0.02ug/ml	**Abnormal**

Table 6.1 Biomarkers Detection From The Sweat

VII. COMPARATIVE ANALSIS OF BIOMARKERS IN BLOOD AND SWEAT

The biomarkers of sodium, potassium, amino acid, lactic acid and heavy metals for one person sample were compared with his blood sample. Comparative analysis shows that the biomarkers in both fluids were approximately equivalent levels. It illustrated that the way of detection of biomarkers in sweat was accurate.

BIOMARKERS	Blood	Sweat	Remarks
	3252 ppm	706.66 ppm	Sodium will be dissolved in blood then only it excrets through the sweat, hence level in the blood is higher than sweat.
POTASSIUM	156 ppm	179.16 ppm	It is approximately equivalent.
LACTIC ACID	10.7 mg/dl	6.66 mg/dl	It is approximately equivalent.

Table7.1 comparative analyses of Bio-fluids

VIII. CONCLUSION

In present work, a simple, inexpensive and noninvasive method was represented for the fortitude of biomarkers in sweat. Sweat employed as a biofluid, additionally to blood and urine, to investigate biomarkers for various disease. Using of analytical instruments the biomarkers in the sweat such as sodium, potassium, amino acid, lactic acid and heavy metals were detected. The detected biomarkers in sweat were compared with the blood.

Biofluids of sweat and blood were compared based on cost, storage time and processing time. The cost of the sweat analysis was low compared to blood analysis. Sample preparation time for sweat was minimum (2 min). There was no need to maintain the sample in particular temperature. Conclusively, sweat analysis was noninvasive method for the detection of biomarkers and favorable bio-fluid for disease identification and analysis of drugs.

REFERENCES

1. G.D.Clayton and F.E. Clayton (eds), Pathy's Industrial Hygiene and Toxicology, 3rd edn, Wiley, New York, 1981, p. 1687.
2. R.A. Goyer and T.W. Clarkson, in: C.D. Klaassen (ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons, 6th edn, MaC-Millan Publishing Company, New York, 2001, p. 8
3. K.Sato,W.H.Kang,K.Saga,andK.T.Sato,"Biologyofsweat glands and their disorders. I. Normal sweat gland function,"Journal of the American Academy of Dermatology ,vol.20,no.4, pp. 537–563, 1989.
4. M.M.Raiszadeh,M.M.Ross,P.S.Russoetal.,"Pro-teomic analysis of eccrine sweat: implications for the discoveryof schizophrenia biomarkerproteins," Journal of Proteome Research, vol. 11, no. 4, pp. 2127–2139, 2012.
5. P. Kintz, A. Tracqui, P. Mangin, and Y. Edel, "Sweat testing in opioid users with a sweat patch,"Journal of Analytical Toxicology,vol.20,no.6,pp.393–397,1996.
6. E. Gallardo and J. A. Queiroz, "The role of alternative specimens in toxicological analysis," Biomedical Chromatography ,vol.22, no. 8, pp. 795–821, 2008
7. Brunet,A.J.Barnes,K.B.Scheidweiler,P.Mura,andM.A.Huestis, "Development and validation of a solid-phase extrac-tion gas chromatography-mass spectrometry method for the simultaneous quantification of methadone, heroin, cocaine andmetabolites in sweat," Analytical and Bioanalytical Chemistry vol. 392, no. 1-2, pp. 115–127, 2008.
8. Huestis,E.J.Cone,C.J.Wong,A.Umbricht,andK.L.Preston, "Monitoring opiate use in substance abuse treatment patients with sweat and urine drug testing," Journal of Analytical Toxicology ,vol.24,no.7,pp.509–521,2000.
9. Liappis N, Kelderbacher SD, Kesseler K, Bantzer P, "Quantitative Study of Free Amino Acids In Human Eccrine Sweat Excreted from Forearms of Healthy Trained And Untrained Men During Exercise," Journal of ApplPhysiolOccupPhysiol 1979;42(4):227-34
10. Lianhui Chen , Shaopu Liu , HongqunLuo and Xiaoli Hu, "Spectrophotometric method for the determination of sodium hyaluronate with basic bisphenylnaphthylmethane dyes", Journal of Chemical and Pharmaceutical Research, 2014, 6(6):1695-1698
11. Humaira Khan , M. Jamaluddin Ahmed , and M. IqbalBhanger, "A simple spectrophotometric method for the determination of trace level lead in biological samples in the presence of aqueous micellarsolutions",Research article, Spectroscopy 20 (2006) 285–297
12. Saimajadoon, Sabihakarim, muhammadroufakram, abidakalsoomkhan,muhammadabidzia,abdulrauf siddiqi,6 and ghulammurtaza, "Recent Developments in Sweat Analysis and Its Applications", International Journal of Analytical Chemistry, 2015, Article ID 164974, 7 pages
13. K. Wilke, A. Martin, L. Terstegen, and S. S. Biel, "A short history of sweat gland biology," International Journal of Cosmetic Science, vol. 29, no. 3, pp. 169–179, 2007.

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14. F. O. Omokhodion and G. W. Crockford, "Sweat lead levels in perons with high blood lead levels: experimental elevation of blood lead by Ingestion of lead chloride," Science of the Total Environment, vol. 108, no. 3, pp. 235–242, 1991.
15. A. K. M. Yousuf, M. Misbahuddin, and M. S. Rahman, "Secretion of arsenic, cholesterol, vitamin E, and zinc from the site of arsenical melanosis and leucomelanosis in skin," Clinical Toxicology, vol. 49, no. 5, pp. 374–378, 2011.
16. Chen WD, Liang Y, Li H, Xiong Y, Liu XD, Wang GJ, et al. Simple,sensitive and rapid LC-ESI-MS method for the quantization of lafutidine in human plasma–application to pharmacokinetic studies. J Pharm Biomed Anal. 2006;41:256–60.
17. Akiba YZ, Kaunitz JD. Lafutidine, a protective H2 receptor antagonist, enhances mucosal defense in rat esophagus. Dig Dis Sci. 2010;55:3063–9.
18. Pan CE, Xu XZ, He HZ, Cai XH, Zhang XH. Separation and identification of cis and trans isomers of 2-butene-1,4-diol and lafutidine by HPLC and LC-MS. J Zhejiang UnivSci B. 2005;6:74–8.
19. Wakabayashi H, Nakajima M, Yamato S, Shimada K. Determination of OLM in rat serum and brain by high-performance liquid chromatography using platinum catalyst reduction and electrochemical detection. J Chromato. 1992;573:154–7.
20. Chintan VP, Amit PK, Anandi DC, Kalpesh TP. Validated absorption factor spectrophotometric and reversed-phase high-performance liquid chromatographic methods for the determination of ramipril and OLM in pharmaceutical formulations. Eur J Anal Chem. 2007;2:3.
21. P. Keerthana, B.G. Geetha, 3 P. Kanmani," Crustose Using Shape Features And Color Histogram With KnearestNeighbour Classifiers", International Journal Of Innovations In Scientific And Engineering Research, Vol. 4, Iss. 9,2017,Pp. 199-203.
22. K.Malarvizhi, R.Kiruba," A Novel Method Of Supervision And Control Of First Order Level Process Using Internet Of Things", Journal Of Advanced Research In Dynamical And Control Systems, Vol. 9, Sp– 6 , 2017, Pp. 1876-1894.
23. Latha.L, Suriya.P And Sindhuja.V.P," Automating The Irrigation System", International Journal Of Pure And Applied Mathematics, Vol.116, No. 11,2017, Pp. 211-219