

BENEFITS OF BIOCHEMICAL PARAMETERS OF SYNOVIAL FLUID AFTER DEATH

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ABSTRACT

Determination of time of death from postmortem changes such as cooling and rigor mortis will be less accurate by progression of postmortem interval (PMI). So, there is another method for estimation of time of death which can withstand putrefactive changes, such as biochemical analysis of body fluids. **Aim:** this study was done for estimation of time passed since death by analysis of synovial fluid parameters urea, creatinine, sodium (Na), potassium (K), calcium (Ca), chloride (Cl), and glucose. **Methodology:** Twenty-six cadavers were examined at postmortem interval: 6-12h, 13-18h, and 19-24h. **Results:** The results of biochemical analysis in different PMI revealed that urea and Na levels had insignificant change. Each creatinine, Cl, and Ca showed significant change and weak correlation with time lapsed, but the correlation with glucose was moderate. **Conclusion:** measurement of glucose + chloride followed by glucose alone in synovial fluid may be helpful in determination of PMI.

Keywords: PMI, synovial fluid, electrolytes, kidney function and glucose

INTRODUCTION

Determination of time passed after death is very important medico-legally in each post-mortem examination. PMI is the length of time from death till corpse discovery (Singh et al., 2006). Many studies pertaining to electrolytes, glucose, glycated hemoglobin, and hormones in several biological fluids have been studied and so support the role of biochemistry as a good tool in forensic pathology (Palmiere et al., 2012)

Synovial fluid biochemical analysis could be helpful in estimating postmortem interval as it can withstand putrefaction and more protected and less prone to burns or atmospheric variations (Sheikh, 2007).

Extensive characters about synovial fluid have been known for at least four decades. Measurement of synovial fluid electrolytes and non-electrolyte concentrations may enhance the

importance of synovial fluid in examination of various modes of death as well as estimation of time passed after death (Arikeri et al., 2013).

AIM OF THE WORK

This work aimed to study the usefulness of synovial fluid biochemical investigations in determination of post-mortem interval.

SUBJECTS & METHODS

1- Subjects

The study was done in medico-legal authority-Minia department in collaboration with forensic medicine and biochemistry departments of faculty of medicine, Minia University from June 2012 to July 2013. Because of higher rate of head injuries or firearm injuries victims that were admitted to medico-legal authority, all corpses were selected from these two types of death. The details regarding the

age, sex, date, time of death, and the history were elicited from admission sheet of the victims.

Exclusion criteria of subjects included:

- (1) Known cases of knee injury.
- (2) Infection of knee.
- (3) Synovial fluid found to be discolored and hemorrhagic.
- (4) Corpse kept in cold storage.
- (5) Unknown cases of time of death.
- (6) Metabolic disorders.

After excluded cadavers, twenty-six cadavers (8 female and 18 male, ages 25-70 years old) who dead from head injuries or firearm injuries, were included in this study.

The synovial fluid samples were taken from:

- 10 cadavers at PMI 6-12h and PMI 13-18h
- 2 cadavers at PMI 13-18h
- 14 cadavers at PMI 13-18h and PMI 19-24h

2- Methods

The synovial fluid was aspirated by puncture the lateral side of suprapatellar pouch or just under the patella

(Madea et al., 2001). Then measurement of:

a- urea: by brithlot method (Tietz, 1995).

b –Creatinine: by Jaffe method (Toora&Rajagopal, 2002).

C- Na, K, and Cl: by ion select electrode (Tietz, 1994).

d- Calcium: by photometric test (Gurder and Zawta, 2001).

e- Glucose: by enzymatic colorimetric method (Keppy et al., 2009)

Statistical analysis were done using software SPSS version 13.

Results:

(I) –Anova test of biochemical values regarding PMI (Table 1):

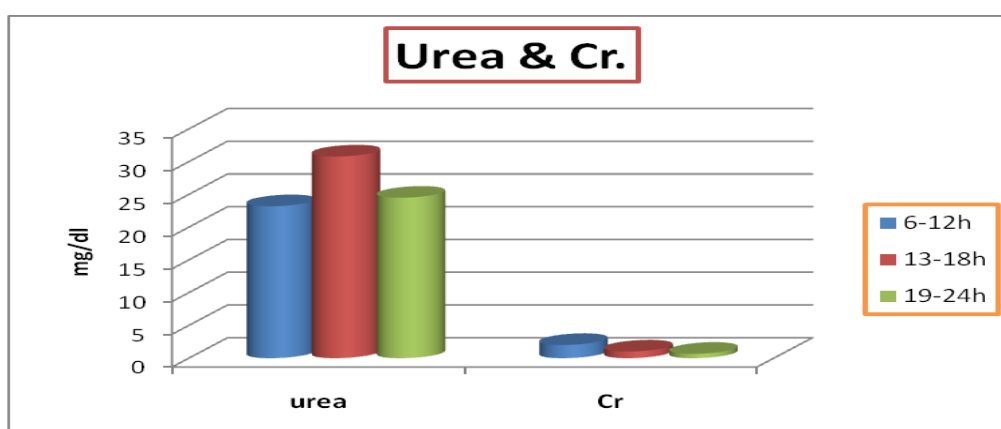
Urea level increased at PMI 13-18h then decreased and this change was insignificant, but creatinine showed significant decrease with time (**fig. 1**). Regarding Na, its level increased at PMI 13-18h then decreased insignificantly, but Cl level had significant decrease with time (**fig. 2**). There was significant change of K level (**fig. 3**). Significant increase in Ca (**fig. 4**), and glucose (**fig. 5**) were shown with time.

Table (1): Anova test for biochemical parameters at different PMI:

	6-12h (I) N = 10	13-18h (II) N = 26	19-24h (III) N =14	P value
Urea: mg/dl				
Range	(9-40)	(14-43)	(12-44)	0.135
M ± SD	23.2 ± 11.61	30.8 ± 9.91	24.5 ± 13.88	
Cr: mg/dl				
Range	(0.6-3.8)	(0.4-2.2)	(0.3-1.1)	0.015*
M ± SD	2.02 ± 1.35	1.02 ± 0.56	0.67 ± 0.37	
Na: mg/dl				
Range	(115-151)	(120-160)	(135-147)	0.146
M ± SD	138.2±11.36	145.07±12.92	140.21±10.22	
K: mg/dl				
Range	(5-6.1)	(3.1-4.7)	(4-5.5)	<0.001*
M ± SD	5.81 ± 0.376	3.65 ± 0.56	4.95 ± 0.67	
Cl: mg/dl				
Range	(105-135)	(90-110)	(95-109)	<0.001*
M ± SD	114.5 ± 9.48	101.96 ± 5.98	100.35 ± 6.81	
Ca: mg/dl				
Range	(4-7.4)	(5-8.7)	(6.9-7.9)	0.004*
M ± SD	5.64 ± 1.26	7.17 ± 1.22	7.20 ± 0.45	
Glucose:mg/dl				
Range	(24-60)	(12-140)	(80-100)	0.001*
M ± SD	37.1±12.84	58.61±47.13	91.42±8.09	

M=mean value SD=standered deviation

P value<0.05 =significant

**Figure (1):** Urea & creatinine at different PMI

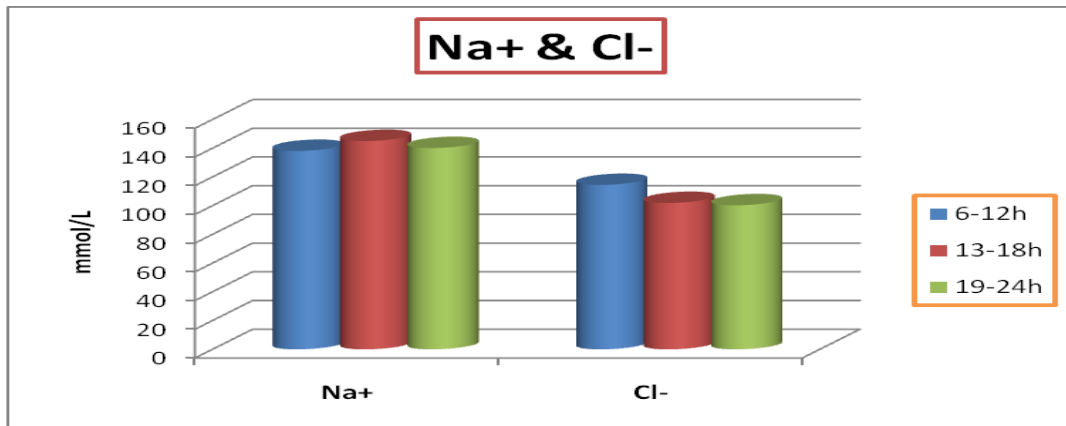


Figure (2): Na&Cl at different PMI

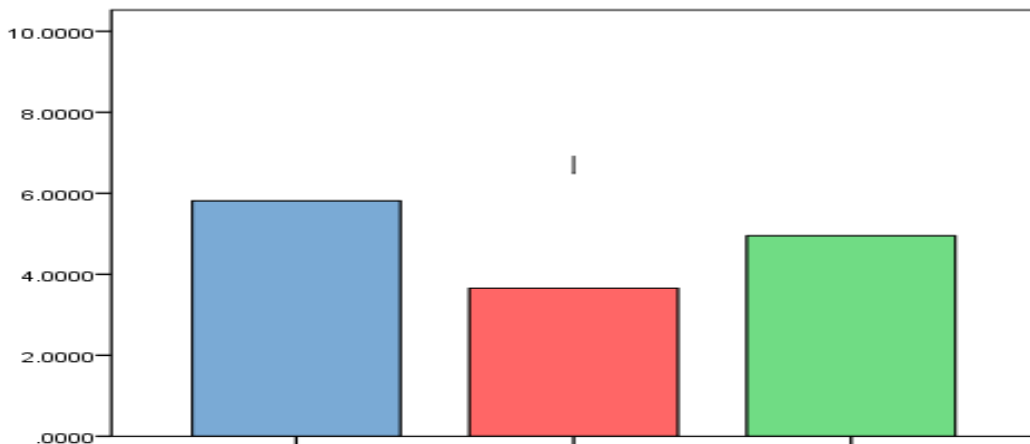


Figure (3): Potassium at different PMI

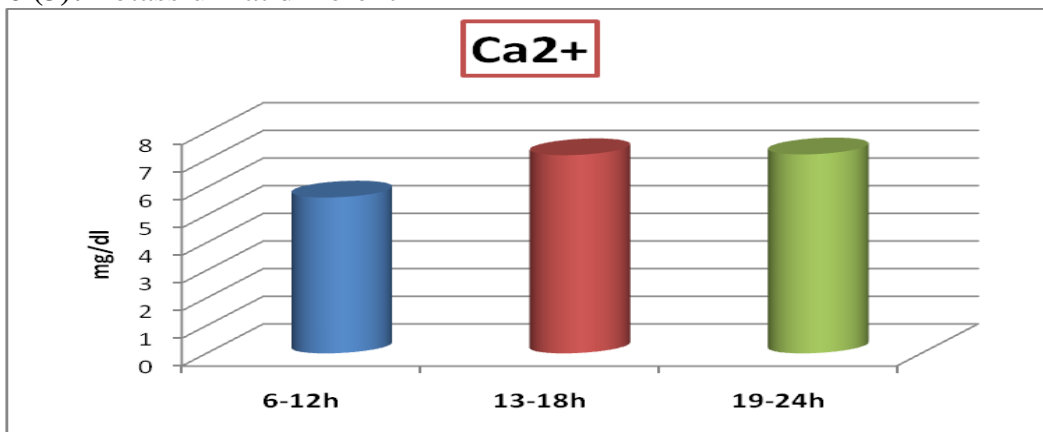


Figure (4): Calcium at different PMI

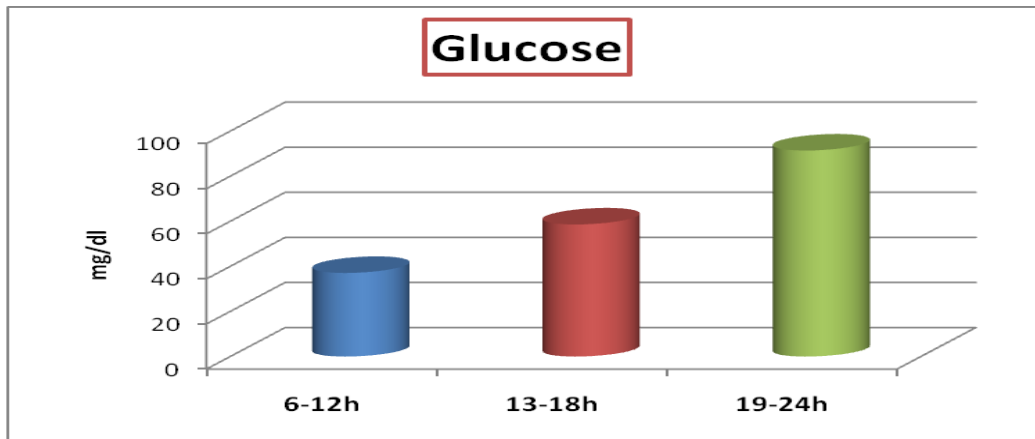


Figure (5): Glucose at different PMI

II- Correlation (table 2), simple regression (table 3-6), and multiple regression (table 7) analysis between cadaveric synovial fluidbiochemical values and PMI:

There were no correlation of urea (fig. 6), sodium (fig.8), and potassium

(fig. 9) with PMI. Significant weak negative correlation was shown in creatinine (fig. 7), and chloride (fig. 10) with time. Positive correlation was noticed in glucose moderately (fig. 12), and calcium weakly (fig. 11).

Table (2): correlation between constituents values of synovial fluid &PMI:

		PMI
Urea	r	-0.022
	P	0.879
Cr.	r	-0.383
	P	0.006*
Na	r	-0.192
	P	0.183
K	r	0.004
	P	0.980
Cl	r	-0.366
	P	0.009*
Ca	r	0.378
	P	0.007*
Glucose	r	0.673
	P	<0.001*

r= correlation

P value<0.05 =significant

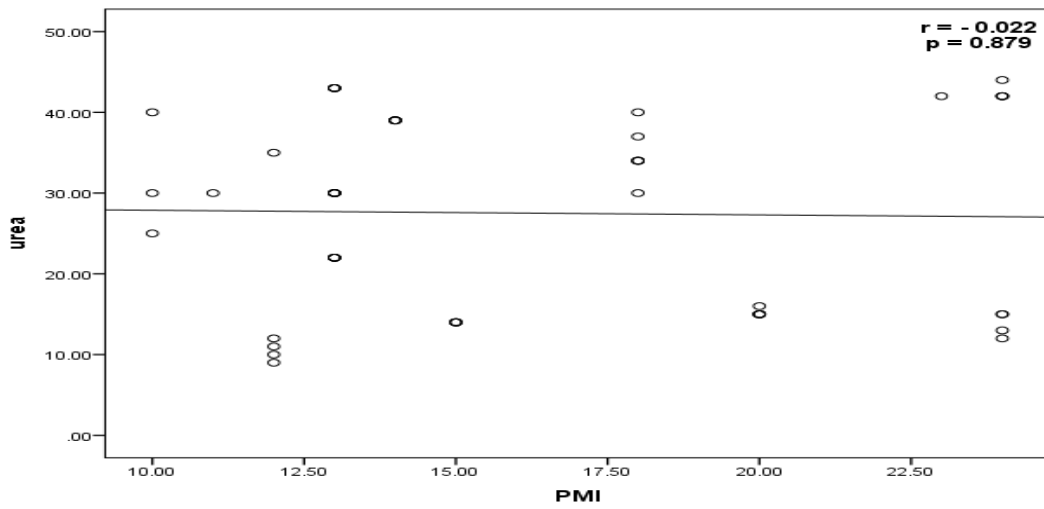


Figure (6): correlation of urea with PMI

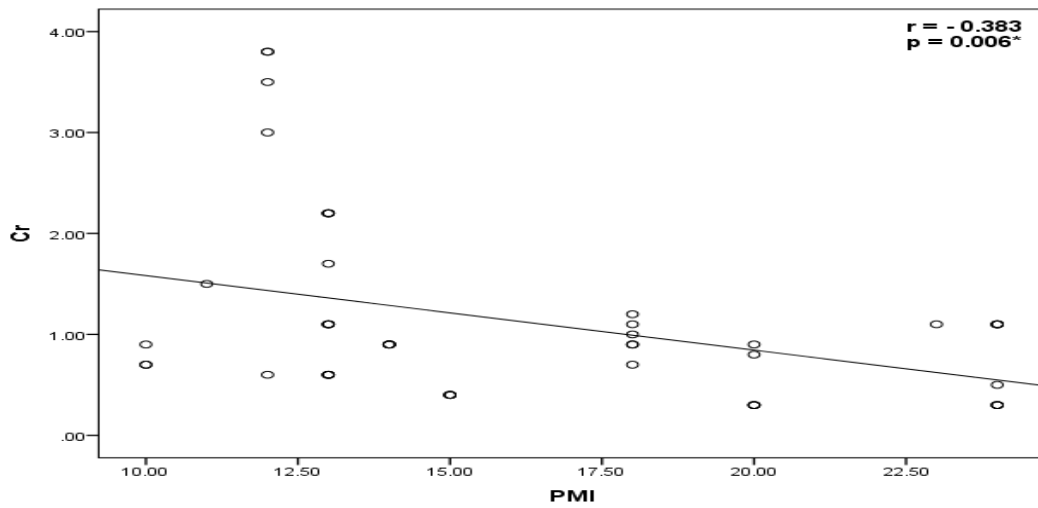


Figure (7): correlation of creatinine with PMI

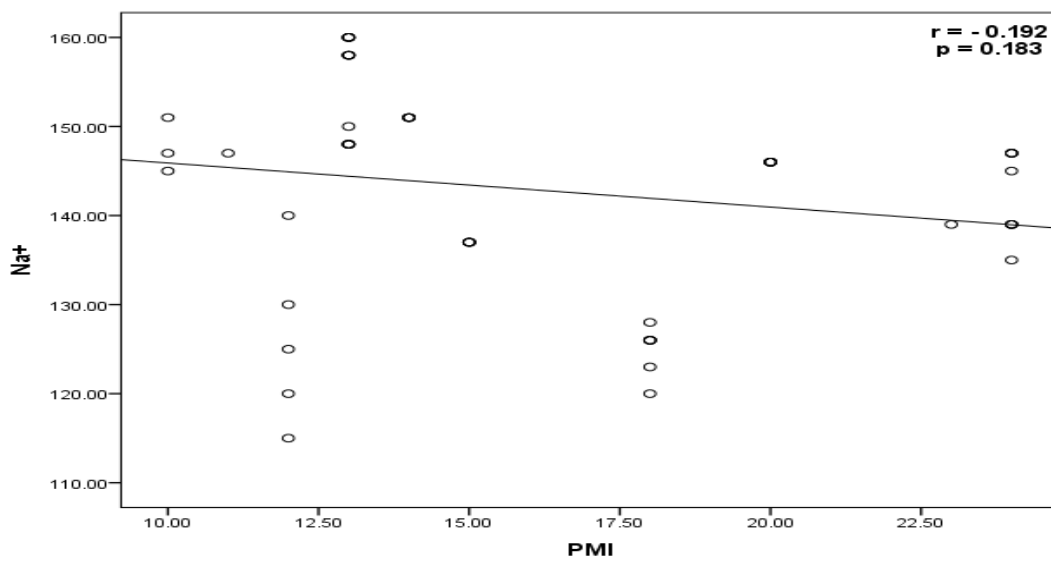


Figure (8): correlation of Na with PMI

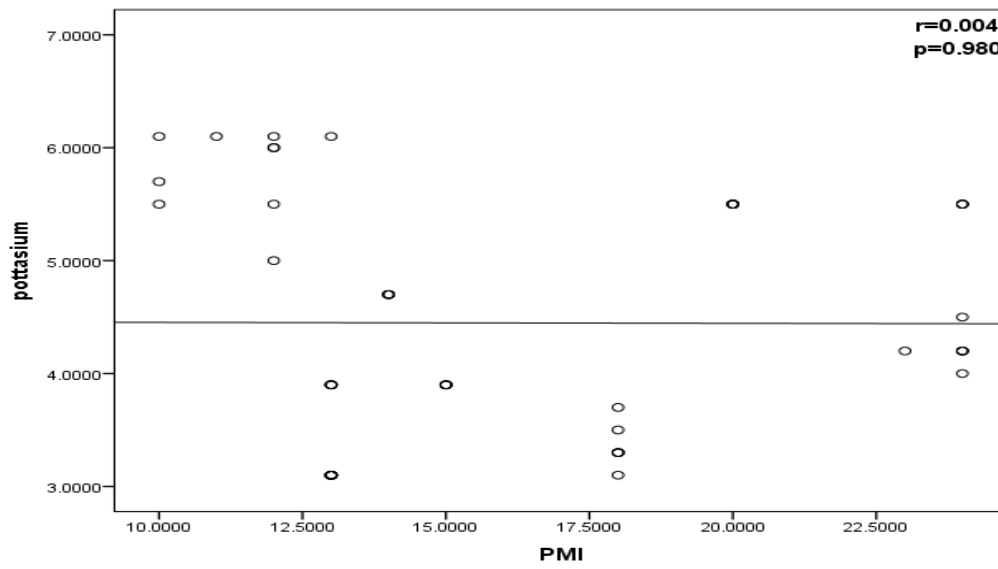


Figure (9): correlation of K with PMI

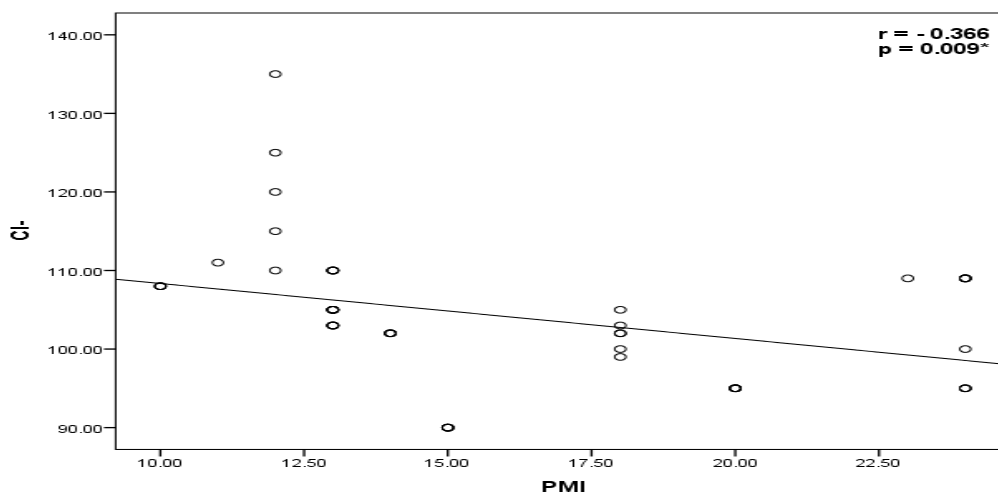


Figure (10): correlation of Cl with PMI

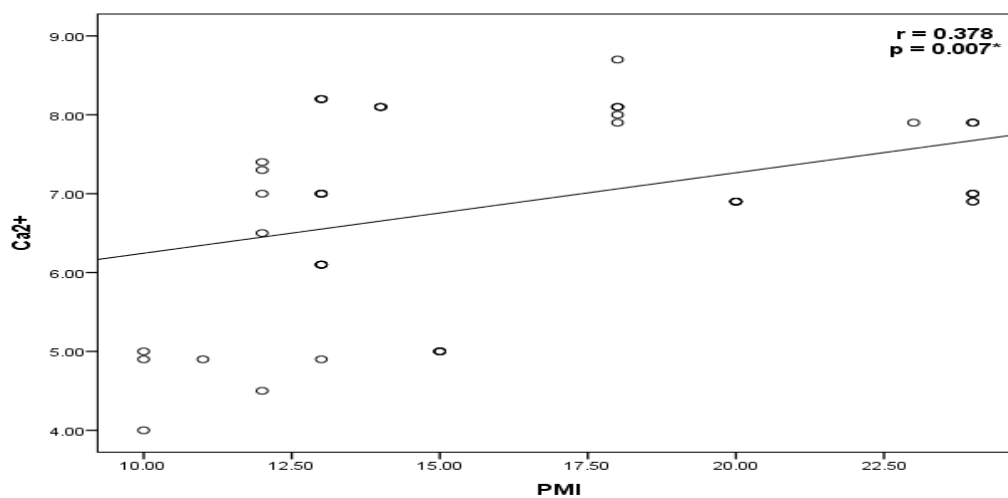


Figure (11): correlation of Ca with PMI

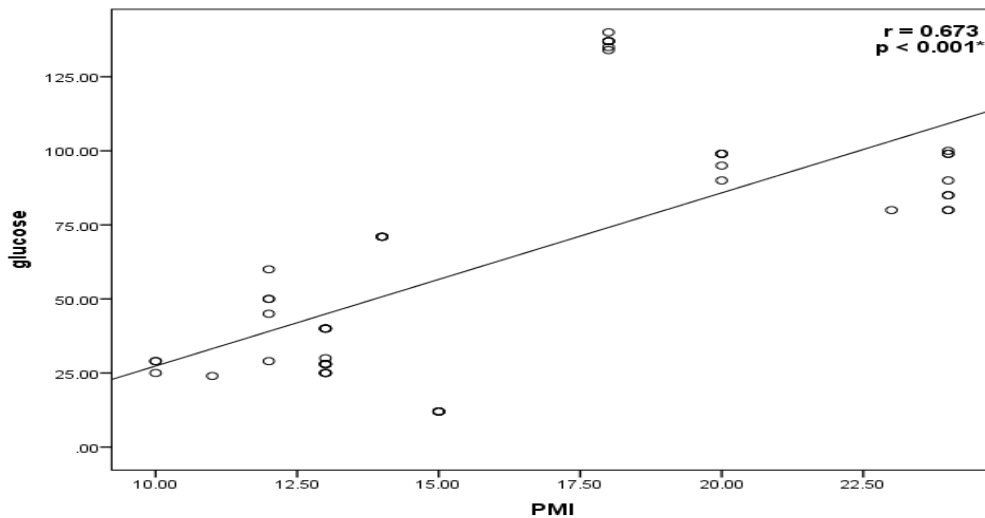


Figure (12): correlation of glucose with PMI

Simple regression analysis by obtain the least square regression equation used to calculate post-mortem interval by:

$$\text{PMI} = \text{B0 (constant)} + \text{B1 (parameter)}.$$

Cr: PMI = (18.421) + (-1.990) Cr level (table 3).

CL: PMI = (36.091) + (-0.191) Cl level (table4).

Ca: PMI = (6.530) + (1.403) Ca level (table5).

Glucose: PMI = (11.255) + (0.078) glucose level (table 6).

The best parameter was glucose because R²=0.453

Table (3): simple regression analysis of creatinine

	R	R²
Cr	0.383	0.147
	coefficients	P value
B0 (constant)	18.421	<0.001*
B1	-1.990	0.006*

Table (4): Showing simple regression analysis of Cl

	R	R²
Cl	0.366	0.134
	coefficients	P value
B0(constant)	36.091	<0.001*
B₁	-0.191	0.009*

Table (5): Showing simple regression analysis of Ca:

	R	R²
Ca	0.378	0.143
	Coefficients	P value
B₀ (constant)	6.530	0.065
B₁	1.403	0.007*

Table (6): Showing simple regression analysis of glucose:

	R	R ²
Glucose	0.673	0.453
	Coefficients	P value
B ₀ (constant)	11.255	<0.001*
B ₁	0.078	<0.001*

Table (7): Showing multiple regression analysis of glucose and chloride

	R	R ²
Glucose & Cl	0.724	0.525
	Coefficients	P value
B ₀ (constant)	26.26	<0.001*
B ₁ glucose	0.073	<0.001*
Cl	-0.141	<0.011*

Multiple stepwise regression analysis by obtain the least square regression equation used to calculate post-mortem interval by:

$$\text{PMI} = \text{B}_0 \text{ (constant)} + \text{B}_1 \text{glucose (its level)} + \text{B}_1 \text{Cl (its level)}$$

$$\text{PMI} = 26.26 + 0.073(\text{glucose level}) + (-0.141) (\text{chloride level})$$

DISCUSSION

It is very important to determine time of death quickly, with accuracy and precision (Nandy,2010). Many researches about postmortem changes studied several biological fluids, and described that the favorite of them were those withstood putrefaction for long time, such as vitreous humor, cerebrospinal fluid, and pericardial fluid (Biswas,2010).

As synovial fluid is more protected and less prone to burns or atmospheric variations, it was thought that the post-mortem chemistry of it might be helpful in estimating post-mortem interval with more accuracy (Sheikh, 2007).

Synovial fluids were aspirated from twenty-six cadavers: at PMI 6-12h (group I), 13-18h (group II), and 19-24h (group III). Synovial fluid was analyzed for measurement of urea, creatinine, Na, K, Cl, Ca, and glucose levels.

This study showed insignificant change of urea and sodium at different postmortem intervals, and that results

are in agreement with Tumram et al., 2011. Synovial fluid Sodium concentration measured by sodium flame photometer, had irregular change and correlation with increasing post mortem interval (Sheikh, 2008).

Creatinine levels had significant decrease and negative correlation with increasing PMI in this research. These results are in contrary with Nishida et al., 2015, who found that the blood creatinine level was high. The suggestion of that controversy may be due to the difference in cause of death which were intoxication and fire in their research but were head injuries and firearm injuries in our research.

Arikeri et al., 2013, concluded that potassium level in synovial fluid had significant change in relation to post mortem period, as the present research detected. The rise of potassium levels were more synovial fluid than vitreous fluid (Madea et al., 2001).

The current study showed significant decrease and negative correlation on comparing the chloride

levels with increasing postmortem interval, and these findings are in accordance with **Tumram et al., 2011**, who used ion selective meter for analysis of chloride in synovial and found significant decrease in its level with time passed since death.

Calcium level showed significant increased and positive correlation with increasing PMI. **Maeda et al., 2011**, found that calcium concentration was immediately increased after death then stable, followed by a final descent of calcium as a result of decomposition. But **Connolly et al., 2016**, found that the serum calcium level after death remains shortly stable then rises slowly until decomposition occur.

In this study, there is significant increased and strong positive correlation on glucose levels with time lapsed. **Madea, 2005**, suggested that the increased the blood glucose level due to postmortem breakdown of carbohydrates in the gastro-intestinal tract.

Contrary to **Trivedi & Narayana, 2015** who demonstrates that the blood glucose level fall in postmortem samples, and that fall is because of the continuation of metabolic reactions and glycolysis after somatic death of an individual.

According to the results of the current study, It can be concluded that measurement the synovial fluid levels of glucose+ chloride ($R^2=0.525$) or glucose alone ($R^2=0.453$) may be helpful in determination of PMI.

PMI = (B0) + (B1) parameter level

= (26.26) + (0.073) glucose level + (-0.141) chloride level

Or = (11.255) + (0.078) glucose level

Finally it is recommended to do more researches about synovial fluid analysis for diagnosis of the cause of death, and to know the effect of pathological process on modification of synovial fluid parameters in determination of postmortem interval.

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فوائد الفحص البيوكيميائي للسائل الزليلي بعد الموت

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الملخص العربي

تحديد وقت الوفاة من التغييرات التي تحدث بعد الوفاة مثل البرودة والتيبس الرمي يكون أقل دقة مع مرور الوقت بعد الوفاة. لذلك، هناك طرق أخرى لتحديد وقت الوفاة والتي يمكن أن لا تتأثر بالتعفن مثل التحليل البيوكيميائي لسوائل الجسم.

لهذا السبب، تم إجراء هذه الدراسة لتحديد وقت الوفاة من خلال قياس معدلات اليوريا والكرياتينين والصوديوم والبوتاسيوم والكلوريد والكالسيوم والجلوكوز في السائل الزليلي لستة وعشرين جثة في فترات: ٦-١٣، ١٢-١٨، ١٩-٢٤ ساعة بعد الوفاة.

وقد كشفت نتائج التحليل البيوكيميائي أن معدلات قيم اليوريا والصوديوم لم يكن لها تغير ذو دلالة احصائية مع مرور زمن الوفاة، ولكن كل من الكرياتينين والبوتاسيوم والكلوريد والكالسيوم والجلوكوز كان له تغير ذو دلالة احصائية مع الزمن. كما ان علاقة زمن الوفاة التوافقية مع الجلوكوز كانت اقوى من الكرياتينين والكلوريد والكالسيوم والبوتاسيوم.

نستنتج مما سبق أن قياس معدلا الجلوكوز+الكلوريد معا يليه معدل الجلوكوز وحده في السائل الزليلي قد يكون مفيدا في تحديد الفاصل الزمني بعد الوفاة