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Evaluation of Catecholamine Levels in Iraqi Female Parkinson's Patients by Using HPLC Technique

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Abstract:

High performance liquid chromatography Reversed–phase (Rp-HPLC) was used to estimate noradrenaline (NA), adrenaline (AD) and dopamine (DA) in the female plasma blood samples of normal volunteer's and Parkinson's patients before and after treatment with levodopa drug. Optimal condition based on ion-pairing has been performed to measure NA, AD and DA on ODS-DB column using 50 mM phosphate buffer mixed with 0.5 mM of 1-octane sulphonic acid: acetonitrile (95:5) at pH = 4.5 and monitored by UV-Visible spectrophotometer at 280 nm.

The chromatographic linearity appears to be linear in the range 0.5-10.0 ppm. The results show that the plasma concentration levels of NA, AD and DA in female normal volunteers were (236(64)), (265(84)) and (692(165)) ng/mL, respectively, whereas the plasma concentration levels of NA, AD and DA in female Parkinson's patients were (277(41)), (175(32)) and (127(42)) ng/mL before treatment with Levodopa drug using general dose of 100 mg/day. After treatment the plasma, the concentration levels become (277(37)), (214(45)) and 359(83)) ng/mL, respectively. Probably, this study has revealed that the non-enzymatic oxidation of NA in addition to DA, promotes a toxic effect by causing an inactivation of the vesicular H^+-ATPase.

***Key Words**: Noradrenaline, adrenaline, dopamine, ion-pairing, biochemical diagnosis, low detection limit.*

تقييم مستويات الكاتيكول امين في مرضى الارتعاش للاناث العراقيات بالاستخدام تقنية الكروموتوغرافية السائل ذات الاداء العالي

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الخلاصة:

استخدمت تقنية كروماتوغرافيا الساتل ذات الاداء العالي الطور العكوس في حسابات تراكيز النورادرينالين، الادرينالين والدوبامين في بلازما الدم للاناث المتطوعين الاصحاء والاناث المصابات بمرض الارتعاش قبل وبعد العلاج بدواء ليفو دوبا. طورت الظروف المثلى لتقنية الايون المشترك لحساب تراكيز النورادرينالين، الادرينالين والدوبامين على عمود ODS-DB بستخدام 50ملي مول من بفر فوسفات الصوديوم ممتزج مع 0.5 ملي مول من الاوكتان سلفونك اسد: اسيتونايترايل (95:5) عند الدالة الحامضية 4.5 وبطول موجي 280 نانوميتر باستخدام مجس الاشعة فوق البنفسجية.

يظهر المخطط الكروماتوغرافي بان الخط المستقيم يكون ما بين 0.5-10.0 جزيء من مليون.

أوضحت النتائج أن مستويات تراكيز النورادرينالين، الادرينالين والدوبامين في بلازما الدم للاناث المتطوعين الاصحاء (236) (64))، (265)(84)) و(692) (165)) نانو غرام/مللي ليتر على التوالي. بينما مستويات تراكيز النورادرينالين، الأدرينالين والدوبامين في بلازما الدم للاناث المصابات بمرض الارتعاش قبل العلاج باليفو دوبا هي على التوالي (277) (41))، (175) (32)) و(127) (42)) ملي غرام/ملي ليتر. أصبحت مستويات تراكيز النورادرينالين، الادرينالين والدوبامين في بلازما الدم للاناث المصابات بمرض الارتعاش بعد العلاج باليفو دوبا خلال جرعة دورية 100ملي غرام/اليوم (277) (37))، (214) (45)) و(359) (83)) على التوالي. من المحتمل أن الدراسة تؤكد على ان الاكسدة الانزيمية للنورادرينالين اضافة الى الدوباميين تودي الى تقليل النشاط الانزيمي لل (vesicular H^+-ATPase).

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Introduction

The Catecholamine is a group of a compound bearing di-hydroxyphenyl moiety[1,2]. Catecholamine; epinephrine (adrenalin), norepinephrine (noradrenalin) and dopamine are synthesized in the adrenal medulla, sympathetic nervous system and the brain. As they influence virtually all tissues are involved with other hormonal and neuronal systems in the regulation of a wide variety of physio-logical processes. The concentrations of catecholamine's and their metabolites are elevated in urine and plasma in several diseases and are of clinical interest, especially in the diagnosis of Parkinson's disease and different types of carcinoma, pheochromocytoma, neuroblastoma and ganglioneuroma[3]. The significances of the biogenic amines are employed as markers of neuroblastoma, stress condition and other autonomic nervous system disorders [4, 5]. Parkinson disease (PD) is marked by a progressive loss of central dopaminergic neurons. The pathogenesis of the disease is related to an oxidative stress in which a cytosolic oxidation of dopamine (DA) seems to play an important role, in the idiopathic as well as in the genetic forms of the disease [6]. The Parkinson disease cause a significant reduction in serum concentration levels of dopamine and adrenaline and such reduction could be recovered when male patients are subjected to levo-dopa drug treatment [7].

Catecholamine is known as main neurotransmitters and adrenaline was discovered as a hormone released from adrenal medulla. Later noradrenaline was established as a main neurotransmitter that is released from peripheral sympathetic nerves. The central neurotransmitter dopamine is known as a precursor of adrenaline and noradrenaline which plays an important role in the metabolism and regulation of sodium ions. Biogenic amines are low molecular weight intercellular messengers and act in chemical signaling [8,9].

Several different analytical techniques, such as spectrophotometry and fluorometry have been used for the determination of catecholamine towards the end of the 1970s the introduction of HPLC with electrochemical detection for the measurement of urinary. Catecho-lamine was considered to be a major advance [10] several modifications to improve the sensitivity of the method in order to obtain reliable results for plasma catecholamine have been subsequently described [11].

However, all these methods are time consuming and cumbersome so that their use is limited to highly specia-lized laboratories. Furthermore, the HPLC methods apply completely different extraction procedures for urinary and for plasma catecholamine prior to column separation. Previously catecholamine and small molecules were separated from plasma proteins by an internal-surface reversed-phase column and analyzed by liquid chromatography - mass spectro-metry using electro spray ionization [12], but by using HPLC and UV detector for simultaneously analysis of noradrenaline and adrenaline methods are unavailable. Previously HPLC with diode array detector and HPLC with fluorometric detector were used but they are involved with multiple steps, complicated, time consuming procedure and sometimes non-reproducible results.

Therefore, the present study was designed to develop methodologies for the simultaneous estimation of catecholamine by RP-HPLC using C-18 column and UV detector [13]. The radioimmunoassay "RIA" and Enzymatic linked immune sorbent assay "ELISA" are two methods which are most commonly used for norepinephrine (NE) and epinephrine (E) analysis. The Catacomb ELISA kit as an alternative to HPLC methods is very useful for clinical applications as well as for basic research where a simple, rapid, accurate and reproducible assay for epinephrine and

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norepinephrine determinations is required[14].

In recent retrospective multicenter studies of usefulness of the different analysts has been investigated. Amongst laboratory assays the measurement of urinary VMA was the most frequently used screening method. However, this was also the method with the lowest diagnostic sensitivity [15,16] and should be considered as obsolete to be replaced by more specific tests [17]. A simple and specific high-performance liquid chromatography method coupled with fluorescence detection (HPLC-FL) has been developed for the simultaneous determination of adrenaline, dopamine and noradrenaline. High performance liquid chromatography is basically a highly improved form of column chromatography. The other major improvement of HPLC over column chromatography concerns the detection methods are given in reference [18]. The new technique was developed as a rapid and sensitive HPLC with fluorescent detection for simultaneous analysis of plasma and urine catecholamine and polyamines without prior treatment procedures via pre-column derivati-zation[19].

The aim of this work is used to investigate the concentration levels of NA, AD and DA in plasma blood samples for Parkinson's females patients compared with normal volunteers and to be used for monitoring the efficacy plasma level of levo-dopa, since is easier as a chemical marker.

Material and Methods:

Apparatus and chemicals

All chemicals used were highest analytical grade obtained from commercial sources and without any further purification. De-ionized distilled water was used for preparation.

Chemicals were obtained as follows; noradrenaline, adrenaline, potassium di-hydrogen phosphate, phosphoric acid and 1-octane sulphonic acid sodium salt from (BDH, England U.K). Dopamine from (Sigma, England U.K), methanol and acetonitrile from Fluka company (ODS-DB) column from (Seplco, England UK).

The chromatographic system was composed from two solvents reservoirs 500 mL fitted with 0.22 µm stainless steel filters at the end of polytriflouroethylene tubes, transferring the mobile-phase from reservoirs to pumps. Two groups of pumps model LC-6A Shimadzu which delivered the mobile-phase (A) and (B) from solvent reservoirs to the mixing cell to create the gradient system program already controlled by SI-6A system controller. The system also involved an injector with 20 µL sample loop model (Reseadyre-7125), (ODS-DB) column 250 x 4.6 mm I.D, thermostatic oven model CTO-6A Shimadzu, UV-Visible detector model SPD-6A and chromatopac unite model R-4A type Shimadzu.

Standard solutions:

10.0 mg of each of noradrenaline, adrenaline and dopamine were dissolved in 50.0 mL of de-ionized distilled water; the standard solutions were filtered, degassed and stored at -20 ^{0}C for further use. The standard solutions were prepared freshly every 30 day.

Mobile-phase:

For catecholamine analysis a stock solution of 50.0 mM potassium di-hydrogen phosphate buffer was prepared by dissolving 3.5 g of potassium di-hydrogen phosphate in 450 mL of freshly prepared de-ionized distilled water. Add 54.0 mg of 1-octane sulphonic acid sodium salt and after completely dissolving diluted to 500 mL in order to obtain buffer pH= 4.5 . The mixture was filtered through 0.22 µm Millipore filter.

Sample preparation:

Five milliliter of blood sample was drawn from vein in to heparinized syringe. The blood sample was immediately transferred in to heparinized polyethylene tube. Cells are removed from plasma by centrifugation for 10 minutes at 1500X g

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using are frigerated centrifuge. Centrifugation for 15 minutes at 2,000X g depletes platelets in the plasma sample. One milliliter of sodium meta- bi-suphate of pH= 8.0 was added to each one milliliter of plasma.

Transfer into polyethylene tube and centrifuged at 1000.0X g for 20.0 min to remove the protein. The samples should be maintained at 28°C while handling. If the plasma is not analyzed immediately, the plasma should be apportioned into 0.5 mL aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze-thaw cycles.

HPLC operation condition:

For the fast, precise and routine method using for separation and determination of catecholamine concentration levels as shown below [20].

Phosphate buffer concentration 50.0 mM containing 0.5 mM 1-octane sulphonic acid sodium salt: acetonitrile (V/V) was 95.0/5.0, buffered at pH= 4.5, column temperature 40.0 ^{0}C, flow rate 1.0 mL/min and wavelength at 280 nm. The typical chromatograms of plasma sample of three components of catecholamine are shown in Figures 1A and 1B.

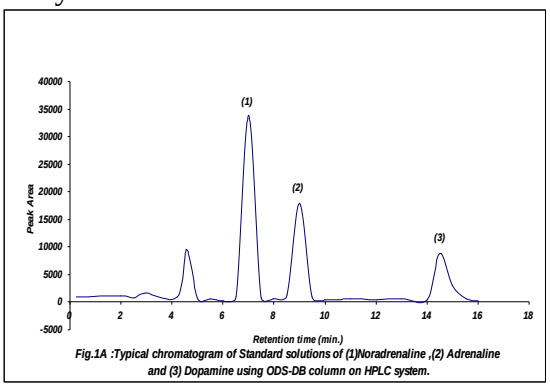

Fig.1A :Typical chromatogram of Standard solutions of (1)Noradrenaline ,(2) Adrenaline and (3) Dopamine using ODS-DB column on HPLC system.

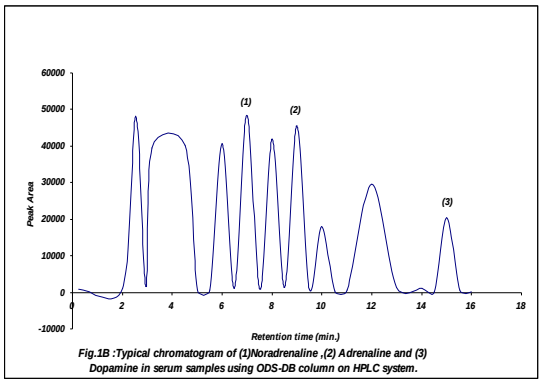

Fig.1B :Typical chromatogram of (1)Noradrenaline ,(2) Adrenaline and (3) Dopamine in serum samples using ODS-DB column on HPLC system.

Linearity:

Linearity was assessed for NA, AD and DA using ODS-DB column in standard solution as well as in biological samples which were submitted to the entire excretion procedure of different concentrations of NA, AD and DA in the range of 0.5-10.0 ppm under optimal conditions which is shown in Figure-2 .

The peak identification of NA, AD and DA in serum samples was performed on the basis of chromatographic retention time and compared by simultaneous injection of standards. The calculated of additional concentrations on the basis of peak area were compared with authored standard in the same range of 0.5-10.0 ppm under the same conditions.

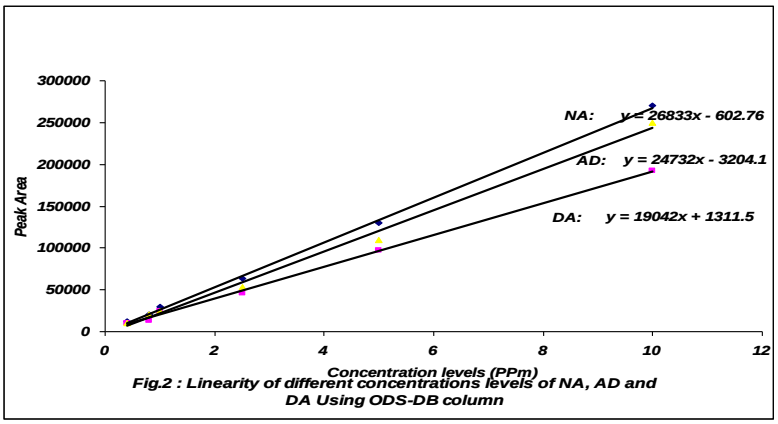

Fig.2 : Linearity of different concentrations levels of NA, AD and DA Using ODS-DB column

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Results and Discussion:

The optimal conditions were used to determine the NA, AD and DA in 17 females of normal volunteer's. The results in Table 1 shows that the concentration levels of NA, AD and DA in females control samples were (236(64)), (265(84)) and (692(165)) ng/mL while the results in Table 2 shows the concentration levels of NA, AD and DA for 11 females Parkinson's patients before treatment were (277(41)), (175(32)) and (127(42)) ng/mL, respectively. Whereas the results in Table 3 shows the concentration levels of NA, AD and DA after treatment with general dose 100 mg/day of levodopa drug becomes (277(37)), (214(45)) and (259(83)) ng/mL, respectively.

The concentration levels of dopamine was highly reduced in Parkinson's patients compare with normal volunteer's due to conversion of dopamine into noradrenaline which was metabolized according to metabolism pathway of adrenaline and noradrenaline. The results in Table 3 shows significant elevation in concent-ration levels of dopamine in females Parkinson's patients under treated with 100 mg/day of levodopa drug due to decarboxylase of levodopa by β-decarboxylase enzyme then converted to dopamine[10]. Histogram in Figure 3 describes the concentration levels of NA, AD and DA for Parkinson's patients before and after treatment with levodopa drug, consequently, improvement in brain neurotransmitter motor will lead to reduce the patient tremor [10].

The Dopamine concentration levels in Parkinson's patients were in the range 0.125 - 0.200 µg/mL which is consistent with other previous study [21].

It is interested to note the concentration levels of dopamine are higher in the females control volunteer's while noradrenaline and adrenaline were slightly changed.

Table-1: Serum concentration levels of NA, AD and DA in (17) females Parkinson's patients before treatment with Levo-dopa drug.

Sample No.	Concentration levels (ng/ml)		
	NA: mean(S.D)*	AD: Mean(S.D)*	DA: Mean(S.D)*
1C	331(2.0)	312(6.0)	450(2.6)
2C	377(2.0)	377(9.6)	661(5.7)
3C	234(2.9)	313(5.7)	630(3.5)
4C	190(7.2)	230(2.6)	454(3.6)
5C	184(3.0)	189(7.2)	508(2.5)
6C	241(5.5)	277(2.6)	779(2.9)
7C	113(2.0)	219(1.7)	650(2.1)
8C	237(2.3)	183(2.5)	539(3.0)
9C	187(2.0)	247(4.2)	631(1.7)
10C	251(1.5)	212(4.6)	567(2.6)
11C	314(2.6)	291(3.5)	887(3.2)
12C	219(3.2)	466(4.7)	774(9.9)
13C	296(2.1)	271(2.0)	900(5.0)
14C	196(2.1)	187(3.1)	845(3.2)
15C	215(2.6)	344(1.0)	917(2.9)
16C	184(1.2)	197(2.5)	895(3.0)
17C	261(3.8)	195(7.8)	819(3.5)
Mean(S.D)**	236(64.7)	265(84.0)	692(165.0)

*Standard deviation of three runs.
**Standard deviation of (17) measurements.

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Table-2: Serum concentration levels of NA, AD and DA in (11) females normal Volunteer's.

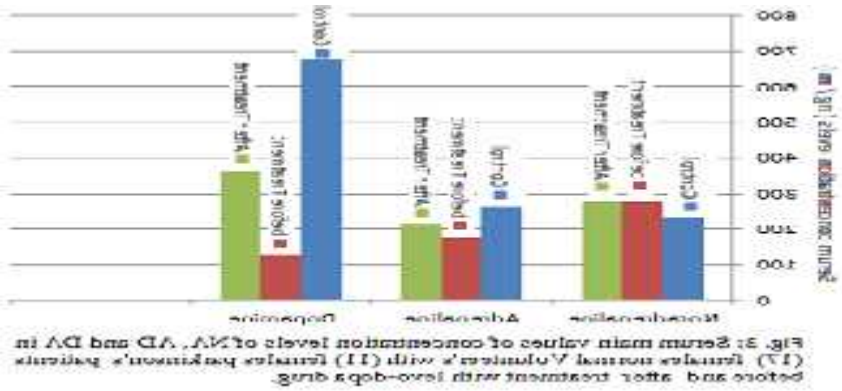
Sample No.	Concentration levels (ng/ml)		
	NA: mean(S.D)*	AD: Mean(S.D)*	DA: Mean(S.D)*
1B	321(1.0)	199(1.7)	89(0.0)
2B	277(0.6)	207(2.1)	109(0.6)
3B	227(2.6)	137(1.0)	121(0.6)
4B	303(1.7)	199(1.0)	208(1.0)
5B	302(3.0)	172(1.0)	213(1.7)
6B	299(1.7)	139(1.0)	113(1.0)
7B	307(1.0)	127(2.6)	102(0.0)
8B	215(1.0)	168(1.0)	113(1.0)
9B	316(1.7)	199(1.0)	113(1.0)
10B	279(1.0)	223(0.6)	113(1.0)
11B	278(0.6)	163(1.0)	103(1.0)
Mean(S.D)**	277(41)	175(32)	127(42)

*Standard deviation of three runs.
** Standard deviation of (11) measurements.

Table-3: Serum concentration levels of NA, AD and DA in (11) females parkinson's patients after treatment with levo-dopa drug.

Sample No.	Concentration levels (ng/ml)		
	NA: mean(S.D)*	AD: Mean(S.D)*	DA: Mean(S.D)*
1A	275(1.7)	188(1.7)	261(2.1)
2A	397(1.0)	220(2.1)	417(1.0)
3A	288(2.6)	297(1.2)	371(1.5)
4A	219(1.0)	168(1.0)	403(3.5)
5A	177(1.7)	233(3.5)	365(4.6)
6A	235(1.0)	172(2.1)	319(2.6)
7A	265(2.6)	169(1.0)	299(1.7)
8A	273(1.0)	179(2.6)	305(1.0)
9A	276(1.0)	192(1.0)	417(2.0)
10A	311(3.6)	229(1.5)	519(2.5)
11A	326(3.6)	291(1.0)	279(0.0)
Mean(S.D)**	277(57)	214(45)	359(83)

* Standard deviation of three runs.
**Standard deviation of (11) measurements.



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Conclusions:

Parkinson disease is characterized by a progressive loss of dopaminergic neurons in the substantial naira pars compact a series of studies indicates that oxidative stress, generated by DA oxidation which is more important .The results that related to dopamine oxidation may cause damage to a wide range of cellular components, including membrane lipids and proteins. Also peripheral noradrenergic fibers are subjected to neuron degeneration in PD.

The present study has revealed that the non- enzymatic oxidation of NA, in addition to DA, promotes a toxic effect by causing an inactivation of the vesicular H^+ -ATPase, the driving force of catecholamine uptake and storage in vitro assay system. In vivo this effect would cause a defect in catecholamine sequestration in synaptic vesicles and increase the amount of cytosolic Catecholamine available for oxidation. This mechanism would add to the effect of lipid damage and α-syncline proto fibril formation [24] which would result in a loss of vesicle membrane integrity and thus a defective catecholamine sequestration in synaptic vesicles. DA, and also NA is easily oxidized in vitro by a number of one-electron oxidants, including, the physiologically important no radical.

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