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CLINICAL EVALUATION OF A NEW FORMULATION OF ^{99m}Tc-TRODAT-1 FOR SPECT IMAGING IN PARKINSON PATIENTS AND VOLUNTEER SUBJECTS

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Keywords:

Brain imaging, Dopamine transporter, Parkinson disease, ^{99m}Tc, ^{99m}Tc-TRODAT-1

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ABSTRACT: This study was conducted to evaluate the clinical usefulness of new developed kit of ^{99m}Tc-TRODAT-1 to differentiate PD(Parkinson disease) from volunteer subjects. The 925-1111MBq (25-30 mCi) ^{99m}Tc-TRODAT-1 samples were reconstituted by boiling water bath method. Fourteen subjects (women = 6, men = 8, age range 24 - 72, mean = 54.4) have been participated in this approach. The control group included (women = 2, men = 2, age = 25-70, mean = 54.75) and the other group included 10 PD patients (women = 4, men = 6, age = 42-72, mean = 54.1) the different stages of PD. Hoehin and Yahr Scale criteria was chosen for clinical evaluation of PD patients. Brain SPECT study has been performed using a single-headed gamma camera 3 hours post intravenous injection. Visual inspection of radiotracer uptake in the striatum and semi-quantitative analyses were performed. The radio-HPLC analysis indicated that the ^{99m}Tc-TRODAT-1 samples were successfully prepared with a radiochemical purity over 90 %. Semi-quantitative analysis demonstrated that the negative relation between the striatal uptake and disease severity has been observed. The sensitivity and specificity of ^{99m}Tc-TRODAT-1 brain imaging to detect PD were 100 % in our investigation. The SPECT brain imaging with a new developed kits of ^{99m}Tc-TRODAT-1can be recommended as a useful modality for diagnosis and evaluation of the severity of PD in clinical practice.

INTRODUCTION: The accuracy of a clinical diagnosis is the most challenging step in every clinical practice. Appropriate treatment is usually the result of an excellent diagnosis with high accuracy.

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Parkinson disease (PD) is a neurodegenerative and movement disorder accompanied by a resting tremor, akinesia (inability to initiate movement), bradykinesia (sluggish movement) and rigidity (muscle resistant to movement) due to progressive degeneration of dopaminergic neurons in the substancia nigra [1]. There is no specific test for PD diagnosis therefore making it sometimes a challenging situation to identify PD, particularly at the early stage of this disorder. PD is usually characterized by the presence of a triad feature of the following symptoms.

These symptoms are rigidity, resting tremor, bradykinesia and postural instability. These clinical manifestations also occur in other neuro-degenerative disorders and by dopamine receptor antagonist pharmaceutical agents. According to the literature, investigations indicated that high rate of misdiagnosis occurred, if the diagnosis was based on only the clinical feature criteria [2, 3]. In addition to the above mentioned factor, motor disturbances are started after a loss of approximately 70 to 80% of the striatal dopamine resources.

Therefore, there is a long latent stage preceding the development of clinical manifestations. It means that the diagnosis of PD based on the clinical feature is not adequate at the present time. These facts indicate that the accuracy of diagnosing PD must be improved, in order to prevent to miss the golden time for medical intervention. The available imaging techniques such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) have high sensitive but are not specific for PD especially in the early phases, when anatomic structures have not been distorted significantly. Radioisotope scintigraphy imaging may be considered as part of the diagnostic procedures to detect PD. The dopamine transporter (DAT) is a protein in the presynaptic membrane on the terminal of dopaminergic projections [4]. This transporter has a pivotal role in the regulation of extracellular dopamine concentration [5]. This molecule has been considered as a marker of dopamine terminal innervations [6]. Degeneration of dopaminergic projections from substantia nigra to the striatum results in loss of DAT [7]. The DAT is localized only on dopaminergic nerve terminals [8].

There is a close relationship between the density of DAT and dopamine in striatum [9]. This criterion has been recommended for the evaluation of PD in clinical practice [10, 11]. Different ligands of DAT have been successful suggested in Positron Emission Tomography (PET) imaging scintigraphy and Single Photon Emission Computerized Tomography (SPECT) radioisotope scintigraphy for the diagnosis of PD [12]. PET scan provides to obtain images with higher resolution than SPECT scintigraphy imaging. SPECT scintigraphy imaging is cheaper and more available than PET scan imaging. Several ^{123}I-labeled DAT SPECT imaging radiopharmaceutical agents based on cocaine or the closely related tropane derivatives have been studied [13-17]. These investigations indicated that the above mentioned modality could be suggested to diagnose the subjects in the preclinical and asymptomatic phase of PD [18]. ^{123}I is the product of cyclotron and relative high expensive.

Therefore, access to cyclotron radioisotopes can impose serious limitations in using this method in routine clinical practice. ^{99m}Tc radioisotope is widely used in nuclear medicine departments. This radioisotope is inexpensive and is readily available. Ligands are labeled by ^{99m}Tc, would be more appropriate for routine use in nuclear medicine practically. Several tropane derivatives were labeled by ^{99m}Tc radioisotope for diagnosing PD [19].

A ^{99m}Tc-labeled tropane derivative, [2-[[-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]-amino]ethanethiolato(3-)-N2,N2´,S2,S2´]oxo-[1R-(exo-exo)] is named (^{99m}Tc-TRODAT-1), has been studied for diagnosing PD. According to the literature, ^{99m}Tc-TRODAT-1 scintigraphy imaging studies have been shown promising results in human studies [20-22]. ^{99m}Tc-TRODAT-1 scintigraphy imaging could open a new path for assessment of the DAT transporter in the brain. Multistep methods have been reported for reconstitution of ^{99m}Tc-TRODAT-1 [23].

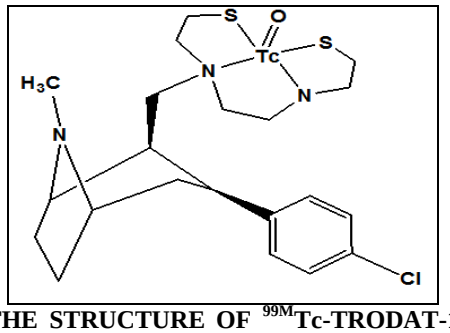

FIG. 1: THE STRUCTURE OF ^{99M}Tc-TRODAT-1 RADIO PHARMACEUTICAL COMPLEX

The reconstitution was then simplified by the formulation containing the lyophilized cold components in a single vial [24, 25]. The solvent extraction and High Performance Liquid Chromatography (HPLC) or C18-Sepack Light cartridge purification have been performed to obtain reliable high radiopharmaceutical purity. The vial must be autoclaved for 30 minutes in order to obtain a radiopharmaceutical purity of larger than 90 % in all above mentioned procedures.

Therefore, ^{99m}Tc-TRODAT-1 scintigraphy imaging is not widely used in clinical practice. Recently, the new lyophilized kit of ^{99m}Tc-TRODAT-1 has been developed, which the reconstitution of ^{99m}Tc-TRODAT-1 radiopharmaceutical kit has been performed due to boiling water bath technique [26, 27]. The method is widely used for the preparation of radiopharmaceutical kits in nuclear medicine. This investigation was conducted to evaluate the potential usefulness of new developed ^{99m}Tc-TRODAT-1 radiopharmaceutical in the assessment of Parkinson patients and volunteer subjects.

MATERIALS AND METHODS: All chemical materials have been purchased from Merck and Fluka. The chemicals and solvents were the highest purity and analytical grade and used without further purification. The new developed freeze-dried kits TRODAT-1 and ^{99}Mo/^{99m}Tc generator have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI). Technetium 99m as sodium pertechnetate was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9 % saline.

Labeling of TRODAT-1 by ^{99m}Tc and quality control: The preparations of ^{99m}Tc-TRODAT-1 complex samples (n=20) have been performed as follow. Briefly, 0.5 ml saline solution was added to an evacuated vial and the mixture was preincubated for 5 minutes at room temperature. The 925-1111MBq (25-30 mCi) freshly eluted Na $^{99m}TcO_4$ in 0.5ml in maximum 0.5 ml saline was added to the above mixture aseptically and put in the lead shield. The content of shield vial was shaken slowly for 30 seconds. The shield vial was heated in a boiling water bath for 30 minutes at 95°C.After heating the shield vial was kept at room temperature for 30 min.

The radiochemical purity and labeling yield of ^{99m}Tc-TRODAT-1 complex samples were determined by ascending instant thin layer chromatography (ITLC) and radio-high performance liquid chromatography (radio-HPLC). ITLC on silica gel (ITLC-SG) was done using ammonium acetate 1M/ acetone in 1/1 ratio for measuring $^{99m}TcO_2$ (^{99m}Tc colloid). Standard 10 cm in length and 2 cm in width strips were used as a stationary phase. The strip was marked with pencil 1 cm (origin) and 0.5 cm (solvent front) from the base. A single spot of ^{99m}Tc-TRODAT-1 complex was applied with a capillary pipette on the 1 cm line. The strips were allowed to dry at room temperature and then placed in an air-tight container. After migration of the mobile phase 1 cm from the top, the strip was dried and cut to ⅓ lower and ⅔ upper pieces. Each piece was counted using a single channel counter with NaI (Tl) detector. The reduced or colloid ^{99m}Tc remained at the point of spotting, while free $^{99m}TcO_4$ and ^{99m}Tc-TRODAT-1 moved to the solvent front.

The final quality control for determination of free $^{99m}TcO_4^-$ and ^{99m}Tc-TRODAT-1 complex was performed with analytical reverse-phase Radio-HPLC on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multi wavelength detector and a flow-through Raytest-Gabi g-detector CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma was used for HPLC. For radionuclide analysis of ^{99m}Tc-TRODAT-1 complex by HPLC, a volume of 10µl of the test solution was injected into the C-18 reverse-phase column and trifluoroacetic acid 0.1%/water (solvent A) and acetonitrile (solvent B) were used as a mobile in following gradient: 0 min A 95% (B 5%), 5 min A 95% (B 5%), 25 min A 0% (B 100%) and 30 min A 0% (B 100%), flow= 1 ml/min **Fig. 2**.

Subject: This clinical study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. Each subject signed an informed consent form based on the guidelines of the above mentioned committee before participation in this investigation. The participants in this approach were divided into main groups A and B respectively. Group A included 4 volunteer people (included women = 2, men = 2, age = 25-70, mean = 54.75) served as control group.

On the basis of the design of this study, none of the volunteers had a history of neuropsychiatric disorders or a family history of movement disorders. They had not taken any medication known to affect the brain dopaminergic system for at least a 6- month before this study. The other group included (included women = 4, men = 6, age = 42-72, mean = 54.1) with various severities of PD. PD was diagnosed according to generally accepted criteria. Patients received neurologic examinations by an experienced neurologist. It was

necessary to display at least two of the following manifestations: resting tremor, rigidity and akinesia with a favorable response to levodopa therapy for clinical diagnosis of PD. All subjects were scored with the Hochn and Yahr Scale (HYS), which ranges from 1 to 4 scales. None of the subjects discontinued their medications or had diet limitations during this approach.

Imaging Protocol: The 925-1111MBq (25-30 mCi) ^{99m}Tc-TRODAT-1 radiopharmaceutical complex has been prepared by the boiled water bath technique and a single bolus injection 925-1111 MBq (25- 30mCi) ^{99m}Tc-TRODAT-1 was administrated intravenously to each participant. All subjects were placed in supine position and their head fixed in a holder. The radioisotope scintigraphy imaging of brain was performed three hours post injection. The images of the brain were acquired by using a single-headed gamma camera (E-Cam, model 2001 Siemens USA) equipped with a high-resolution low energy collimator. Acquisition parameters were as follow: SPECT, matrix 128*128, projection 64, each projection equal 25 second. The filter back projection and iterative methods have been applied for reconstruction.

According to the observer's experience the quality of images has been obtained by an iterative method were more suitable than the quality of images obtained by filter back projection. Therefore, the iterative method has been used for the reconstitution of the images. Two criteria have been chosen for interpretation of the radioisotope scintigraphy images. First, the specific uptake in striatum in comparison to the nonspecific uptake of radiotracer in occipital lobes (OL) has been considered visually. OL has been chosen as a reference area because of the low density of DAT there and the capability to compare data between the striatum and the OL from the same slice.

Although different reference areas have been used for ^{99m}Tc-TRODAT-1 scintigraphy imaging, calculating the ratio of striatal activity to OL activity is simple and easy to apply in clinical investigations. Second, the SPECT images were analyzed by a semi-quantitative study using available commercial software. Three slices with the highest striatum activity were selected. The regions of interest were created over the striatum nucleus of each hemisphere. The regions of interest of OL were created in the same way and served as a background area. The specific of radiotracer uptake was calculated by the absolute values by subtracting the mean counts per pixel in the OL from the mean counts per pixel in each hemisphere and dividing the result by the mean counts per pixel in the background. In other words, the equations are | (Hemisphere $_{right}$- OL)|/ OL and |(Hemisphere $_{left}$- OL)|/OL. The mean specific uptake of the striatum nucleus of each hemisphere in volunteers and PD subjects was calculated. All images were interpreted by four independent nuclear physicians who were unaware of the clinical status of the subjects and their final opinion was achieved by consensus. The images were interpreted both visually and by semi-quantitative analysis **Fig. 3**.

Statistics: The calculations of means and standard deviations were made on Microsoft Excel. The data were shown as the mean ± SD. The student t-test was used to determine statistical analysis. Statistical significance was defined as $p<0.001$.

RESULTS: The ^{99m}Tc-TRODAT-1 radio pharmaceutical complex and two main radio chemical impurities have been produced during radio labeling procedure of new developed TRODAT-1kit with ^{99m}Tc radioisotope due to boiling water bath method. These main radio chemical impurities were $^{99m}TcO_4^-$and $^{99m}TcO_2$.The amount of desired complex and $^{99m}TcO_4^-$ could be measured by Radio-HPLC analysis. The retention times of $^{99m}TcO_4^-$ and ^{99m}Tc-TRODAT-1 complex were approximately 4.41and 17.91 minutes, respectively **Fig. 2**. The areas under the peaks of $^{99m}TcO4^-$ and ^{99m}Tc-TRODAT-1 complex were related to their concentrations.

The amount of $^{99m}TcO_2$ was measured by ITLC analysis. In our approach the yields of ^{99m}Tc-TRODAT-1complex and $^{99m}TcO_4^-$ were 96.32 ± 1.15 % and 3.68 ± 0.8 (n=20) respectively. The results obtained by the ITLC study showed some $^{99m}TcO_2$ impurity which remained at spotting origin were less than 2.65% in this study. This matter demonstrates the successful reconstitution of ^{99m}Tc-TRODAT-1 complex due to a boiling water bath method. The radioisotope scintigraphy imaging has been performed 3 hours after 925-1111MBq (25-30 mCi) ^{99m}Tc-TRODAT-1 injected intravenously.

The scintigraphy images have been performed by single-headed gamma camera. It has only provided to investigate the accumulation of radiotracer in the striatum. It was not possible to assess the radiotracer uptake in the putamen and caudate nucleus. All images were interpreted both visually and by semi-quantitative analysis.

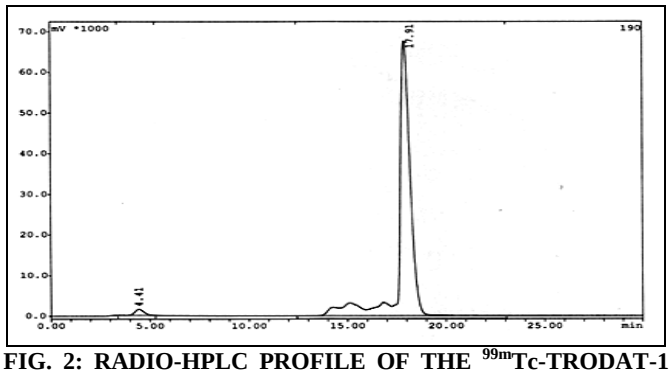

FIG. 2: RADIO-HPLC PROFILE OF THE ^{99m}Tc-TRODAT-1 COMPLEX AFTER LABELING WITH ^{99m}Tc. The retention time of $^{99m}TcO_4^-$ is 4.41 min and for ^{99m}Tc-TERODAT-1 radiopharmaceutical complex is 17.91min.

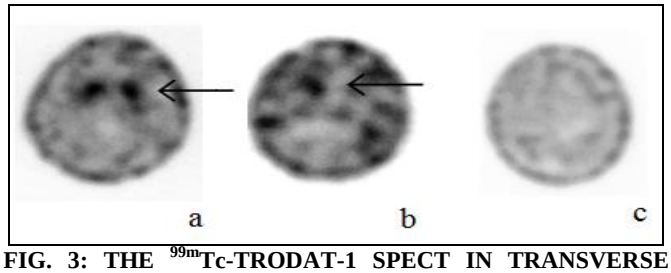

FIG. 3: THE ^{99m}Tc-TRODAT-1 SPECT IN TRANSVERSE VIEWS HAVE BEEN OBTAINED THREE HOURS AFTER INTRAVENOUS INJECTION a: an age-control volunteer b: asymmetric striatum uptake was found in the early stages of PD c: significant loss of radiotracer uptake in the whole striatum was observed in advanced PD.

We observed that radioactivity accumulated in the striatum region of each subject. The quality of images was appropriate for scintigraphy imaging. On SPECT images, a suitable contrast of radioactivity between the striatum and adjacent brain tissue was observed in group A. Three subjects in group A were older than 40 years and had diabetes and consumed medication for their disease. One volunteer subject was younger than 40 years and free of any disease. The specific uptake of ^{99m}Tc-TRODAT-1 in the right and left sides of striatum were 0.5 ± 0.014 and 0.545 ± 0.06 respectively in the group A. The uptake of ^{99m}Tc-TRODAT-1 was symmetric in the control group.

Significant differences in the specific uptake of radiotracer were not observed among volunteers **Fig. 3a**. All subjects in group B were older than 40 years. They were at different stages of Parkinson disease. In addition to PD, four subjects had hypertension and diabetes (3 hypertension and 1diabetes). They took their medication in addition to PD's therapy. The specific uptake of radiotracer in the right and left sides of the striatum were 0.15 ± 0.13 and 0.13 ± 0.14 in the group B respectively. A continuous reduction in specific striatal uptake of radiotracer with increasing disease severity was observed in PD. The reduction of radioactivity was most pronounced to the dominant symptomatic side.

To discriminate PD from volunteer could be performed easily by viewing the images. The accumulation of radio tracer was dependent to the stage of PD. The radiotracer uptake was asymmetric for the participants at the early-stage of PD **Fig. 3 b** but a marked decreased in radiotracer uptake in the striatum was easily observed at the end-stage of PD **Fig. 3 c**. One subject with clinical manifestations of tremor and rigidity was recently diagnosed as PD. She started taking levodopa in order to control her complications. The SPECT images indicated that radiotracer uptakes in the right and left sides of striatum were normal. Levodopa therapy could not be alleviated her complications.

Further medical examinations were meticulously undertaken at the clinic by neurologist. The medical evaluation attempts indicated that she has a psychogenic tremor. Therefore, the levodopa therapy was discontinued and other medical interventions have been considered. No considerable adverse reactions were observed in either the control group or PD subjects during the imaging procedures or during a 3-month follow-up period. The data obtained from this study demonstrated that the scintigraphy imaging with the newly developed ^{99m}Tc-TRODAT-1 was not shown to interact with the other drugs that the participants consumed. It is not necessary to consider any diet restrictions for scintigraphy imaging. Imaging with new developed radiotracer was only dependent to the appropriate functional DAT in the brain dopaminergic projection system. The sensitivity and specificity of The newly developed radiotracer to detect PD was 100 % for the subjects examined. The demographic characteristic of all participants and their imaging findings has been shown in **Table 1**.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF ALL PARTICIPANTS IN THIS STUDY AND THEIR IMAGING FINDINGS ARE DEMONSTRATED

O	Group	Sex	Age (Y)	DD (Y)	HYS	Clinical Manifestations	Antiparkinson therapy	Visual 99mTc-TRODAT-1 analysis	Uptake R	Uptake L	Concomitant disease	Other drug therapy
1	A	M	25	-	-	-	-	Uptake bilateral	0.51	0.51	-	-
2	A	F	67	-	-	-	-	Uptake bilateral	0.48	.49	Diabete, Hypertension	Captopril, Insulin NPH, Metformin
3	A	M	70	-	-	-	-	Uptake bilateral	.51	.56	Diabete	Metformin, Glibenclamid
4	A	F	57	-	-	-	-	Uptake bilateral	0.5	0.62	Diabete	Metformin
5	B	M	52	3	1	Rigidity, Tremor more on R	Levodopa Pramipixole	Decreased uptake more on L	0.21	0.15	-	-
6	B	M	42	2	3	Bradykinesia, Rigidity, tremor Bilateral	Levodopa Amantadin	Decreased uptake bilateral	0.01	0.07	-	-
7	B	F	74	1	1	Rigidity, Tremor more on L	Levodopa Amantadin	Decreased uptake more on R	0.04	0.11	Hypertension	Captopril
8	B	M	56	3	2	Bradykinesia, Rigidity, Tremor more on R	Levodopa,	Decreased uptake more on L	0.16	0.07	Hypertension	Losartan
9	B	F	43	Less than one month	Special normal	Rigidity, Tremor	Levodopa	Uptake bilateral	0.43	0.47	-	-
10	B	M	67	3	3	Rigidity, Tremor, Mask face,	Levodopa, Pramipixole	Decreased uptake bilateral	0.2	0.24	Diabete	Metformin
11	B	F	42	2	3	Bradykinesia, Rigidity, tremor Bilateral	Levodopa, Pramipixole	Decreased uptake bilateral	0.00	0.02	-	-
12	B	F	57	3	3	Bradykinesia, Rigidity, tremor Bilateral	Levodopa, Amantadin	Decreased uptake bilateral	0.1	0.05	Hypertension	Atenolol
13	B	M	48	3	2	Rigidity, Tremor more on R	Levodopa	Decreased uptake more on L	0.27	0.03	-	-
14	B	M	60	3	3	Bradykinesia, Rigidity, tremor Bilateral	Levodopa	Decreased uptake bilateral	0.07	0.07	--	-

Group A: Volunteer subjects, Group B: Parkinson disease patients, DD: Disease Duration, HYS: Hochn Yahr Scale, L: Left side, R: Right side, Uptake R: Radiotracer uptake in right hemisphere, Uptake L: Radiotracer uptake in left hemisphere.

DISCUSSION: PD is the second most common neurodegenerative disorder after Alzheimer's disease [28, 29]. Different mechanisms have been suggested to be involved in the pathogenesis of PD. An easily and reliable technique for the preferentially discrimination of PD is still unavailable. Several clinical criteria have been recommended and established [30 - 32]. The most recent one has classified PD as possible, probable and definite PD. The presence of ubiquinated protein deposits in the cytoplasm of neurons, called Lewy bodies in the pathologic finding is required in order to diagnosis of definite PD [33, 34]. Lewy bodies are also present in many other disorders [35, 36]. Practical laboratory assistance is highly desirable to discriminate PD.

Dopamine belongs to the catecholamine family and has a crucial role as a neurotransmitter and neuroendocrine transmitter in the brain and other organs of humans. Different kinds of nervous disorders are associated with dysfunctions of the dopamine system. The DAT protein molecule is one of the regulatory mechanisms which pumps the dopamine back to the presynaptic neuron projections through the dopamine transporters. The density of transporter is dramatically declined in patients with Alzheimer and Parkinson diseases [7, 37]. Neuro-imaging techniques using ^{18}F-Dopa for PET imaging have been considered as a gold standard for the evaluation of the striatal dopaminergic neurons in PD [2, 38]. The dopamine transporter is a protein located on the membrane of the dopamine terminal in striatum.

A compensatory down regulation of the DAT in the striatum in PD is occurred. This phenomen indicates that the functional imaging using the DAT ligand is more sensitive than ^{18}F-Dopa in diagnosing the degeneration of dopaminergic neuron projections. Different ligands have demonstrated high affinity for the DAT molecule.

These ligands have shown potentially characteristics to label by different radioisotopes. It could be provided for PET and SPECT imaging for dopamine transporters in nervous system. Ligands such as ^{11}C-CFT for PET imaging and ^{123}I-βCIT for SPECT imaging have shown promising results to detect PD. These radioisotopes are produced by the cyclotron. This requirement seriously limits their clinical applications practically. ^{99m}Tc radioisotope is widely used in diagnostic procedures practically.

Its popularity is mainly to the matter that the radioisotope can be readily produced by ^{99}Mo/^{99m}TC generators. It has the ideal γ-ray energy (140 keV) which is suitable for gamma camera detection. In addition to the above mentioned factors, the physical half-life is compatible with the biological localization and residence time required for radioisotope scintigraphy imaging. The various complexes of ^{99m}Tc may be formed by interactions between donor electron atoms and the empty orbital of reduced ^{99m}Tc. The structure of the ligand must have electron donors such as oxygen, nitrogen and sulfur in order to form bonds between the ligand and ^{99m}Tc. ^{99m}Tc radioisotope in the form of pertechnetate is present in the elution solution obtained from ^{99}Mo/^{99m}TC generator. The reducing agents such as stannous chloride are commonly used to reduce the ^{99m}Tc radioisotope from +7 to a lower valence state which can react with ligands. The labeling of some ligands with ^{99m}Tc is performed at the room temperature like ^{99m}Tc-MDP.

The process of labeling various ligands such as MIBI with ^{99m}Tc is undertaken by heating. The water boiling bath method is commonly used for the reconstitution of radiopharmaceuticals. TRODAT-1 has been proven to selectively bind to DAT and to label it by a ^{99m}Tc radioisotope. ^{99m}Tc-TRODAT-1 combines the advantages of ^{99m}Tc labeling and avidity bonding TRODAT-1 to the DAT transporter for visualizing the striatum by scintigraphy imaging. The reconstitution of ^{99m}Tc-TRODAT-1 has been performed by a multistep method. The labeling was then simplified by the formulation containing the lyophilized cold components in a single vial. The solvent extraction and HPLC separation must be used in order to obtain high radiopharmaceutical purity. The vial should be autoclaved for 30 minutes to obtain the suitable radiochemical purity. The method is highly desirable to develop for the reconstitution of radiopharmaceutical that autoclave facility is not used in the preparation of radiotracer in nuclear medicine departments for a widespread clinical application of ^{99m}Tc-TRODAT-1.The labeling of new developed freeze-dried kits was completed on boiling water bath in order to obtain high specific activity.

The newly developed formulation kit contains TRODAT-1, tricine, mannitol and stannous chloride. The dose of TRODAT-1from 200 µg used in the multistep kit has been reduced to 10 µg which was equal with the amount that was used in the one vial kit formulation [26, 27]. Although, this amount of ligand is very low, but it is sufficient to perform scintigraphy imaging. The main reason for the reduced ligand dose is related to this matter that the non-labeled free ligand cannot readily cross the blood brain barrier as well as ^{99m}Tc-TRODAT-1.

Therefore, decreasing the ligand dose used in the formulation is highly desirable. The new developed kit was used for scintigraphy imaging in our approach. The quality of striatal images was appropriate for assessment by both visual interpretation and semi-quantitative analysis. The results from our study indicated that a considerable reduction of striatal uptake of ^{99m}Tc-TRODAT-1 in PD has been observed. This finding is consistent with previous investigations and suggests that ^{99m}Tc-TRODAT1 can be considered as a sensitive marker of the presence and severity of PD. A suitable agreement has been observed between the visual inspection and the semi-quantitative analysis of the presence of PD. Therefore, visual inspection of ^{99m}Tc-TRODAT-1 scintigraphy imaging might be adequate to discriminate PD from healthy subjects. We found a good correlation between the striatal uptake and the severity of PD. Asymmetrical striatal uptake was observed in the early stages, whereas a remarkable reduction of radiotracer uptake in the whole striatum was observed in advanced PD. This approach is one a few studies that used a single-headed gamma camera. For this reason, we could only find a good contrast between the striatum and the background. The caudate and putamen could not be identified clearly.

Therefore, nigro dopaminergic projections to the striatum are considered as a target in PD and volunteer subjects. The promising results with single headed gamma camera have been reported in distinguishing PD from healthy subjects and also in assessing the severity of PD by ^{123}I β-CIT scintigraphy imaging [39].The double-headed and triple-headed gamma cameras are preferred for brain SPECT studies.

The imaging by double-headed or triple-headed gamma cameras is more comfortable than a single-headed gamma camera for the PD patients because the time of the study is considerable reduced. The artifacts could be created in radioisotope imaging due to the head movement of PD patients. For this reason, monitoring the subjects during radioisotope imaging particularly with a single-headed gamma camera was crucial because rotational motion artifacts were difficult to identify. The striatal radiotracer uptake in volunteers was identical approximately. This finding indicated the decline of DAT density is not age dependent. It is contrary to the results were previously reported [22, 40].

The effect of age on DAT density is still controversial. Muller *et al*., reported that age has not significantly influenced the relative or absolute striatal uptake in PD patients [41]. Mozley *et al*., reported that there is not a linear relationship between age and DAT density [42]. This discrepancy can be explained by differences in the method of radiotracer preparation, in models of technical equipment, in the reference areas and biological redistribution after administration.

The above mentioned factors may have contributed to some differences between our achievement and those of previous investigations. The dose of ligand in the newly developed formulation is very low. The avidity of ligand to tag the DAT molecule is high. Therefore, the ^{99m}Tc-TRODAT-1 radiotracer accumulates in striatum more than the other parts of the brain where DAT is not present. If the functional DAT molecules are available, the striatum region can be visualized easily. The new developed kit has the following advantages. First it can be labeled by ^{99m}Tc radioisotope. Second the labeling process can be performed by the boiling water bath method and this technique is widely used in nuclear medicine departments.

Third, it is not necessary for PD patients to discontinue their medication during scintigraphy imaging. Fourth any diet restrictions should not be considered. SPECT study with ^{99m}Tc-TRODAT-1 radio pharmaceutical provides images with good quality, have high target to non-target ratio and demonstrate patterns of loss DAT in PD patients. It can be recommended for preferentially diagnosis of PD and assessing patient status.

CONCLUSION: The imaging with labeled ligand of DAT can be provided as an alternative technique to evaluate the dopamine neuronal function. The data obtained from this study demonstrated that the brain imaging with a newly developed kit of ^{99m}Tc-TRODAT-1 is readily available in clinical practice to discriminate PD patients. The development of a single freeze-dried kit with the advantages of labeling with ^{99m}Tc radio isotope, readily reconstitution and inexpensive procedure can be recommended to patients who are suffering from PD.

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