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Abstract:

**Background:** $^{99m}$Tc-HMPAO brain scan is well-known nuclear medicine test for the detection of regional brain blood flow. As we know that diabetes can affect the blood flow in certain organs such as heart, kidneys and eyes. Brain also can be affected. Therefore, our objective of this study was to investigate the role of $^{99m}$Tc-HMPAO brain scan in detecting the early vascular changes in brain in early diabetes. **Methods:** There were two groups of male Wistar rats (one control and one streptozotocin (STZ) induced diabetic group). Each group contained 6 rats. Rat brain imaging using gamma camera was performed for each group after 0.5 (control), 2, 4, and 24 h post 129.5 MBq injection of $^{99m}$Tc-HMPAO. Data processing for brain scan was done by drawing a region of interest (ROI) circulating the brain (B) and a ROI around the soft tissues around the neck of each rat as background (BKG). Brain $^{99m}$Tc-HMPAO uptake minus background (net brain counts; NBC) in control rats were studied first and expressed as the mean ± standard deviation (mean±SD). Then NBC in diabetic-STZ rats were studied. **Results:** The NBC in control group (mean±SD) was:

- $67766.83±10405.19$, $49439.67±6960.06$, $37080.83±5459.16$,
- $2017.00±302.72$ counts per sec (cps) at 0.5, 2, 4 and 24 h respectively. The NBC in STZ group was:
- $241296.83±3548.81$, $148215.17±2316.45$,
- $111254.17±1682.65$, $6817.83±76.36$ cps at 0.5, 2, 4 and 24 h respectively. A statistical comparison (Student's $t$-test) between the NBC controls and the
NBC STZ diabetic group at each time point was determined. There was highly significant difference between control and STZ rats at each time point (P < 0.0001). **Conclusion:** $^{99m}$Tc-HMPAO brain scan has an important role in the diagnosis of early diabetes in brain rat as early as one week duration.
Background

Technetium 99m-hexamethylpropylene amine oxime (\(^{99m}\)Tc-HMPAO) brain scan is well-known nuclear medicine test for the detection of regional brain blood flow. Diabetes can induce several pathophysiological complications such as vascular changes in certain organs such as heart, kidneys and eyes. Brain also can be affected. By its nature nuclear medicine is suited for functional investigation of various processes. For example in the podiatric literature, nuclear medicine imaging showed assistance in the management of the diabetic foot [1].

The role of nuclear medicine brain imaging in the detection of diabetes was reported using Positron Emission Tomography (PET), fluorine-18 fluorodeoxyglucose (\(^{18}\)F-FDG), to assess brain glucose metabolism [2-3] in diabetic patients. Only four studies examined cerebral perfusion in diabetes with SPECT [4-7]. Three of them were involved in detecting type 1 diabetes and the fourth was involved in detecting type 2 diabetes and all were done in patients.

\(^{99m}\)Tc-HMPAO is the most common radiotracer for SPECT and planar brain imaging; it is a lipophilic imaging radiotracer that crosses the blood brain barrier (BBB). \(^{99m}\)Tc-HMPAO accumulates in the brain as proposed mechanism is through its intracellular conversion from a lipophilic form to more hydrophilic form within the brain parenchyma [8]. Under most
pathological conditions, blood flow is coupled to metabolism and therefore brain images give an indication of the functional status of the brain.

By our knowledge, no study in the literature investigated $^{99m}$Tc-HMPAO brain uptake and the role of $^{99m}$Tc-HMPAO brain imaging in detecting the vascular changes in early diabetes in rats. Therefore our objective of this study was to investigate the role of $^{99m}$Tc-HMPAO brain scan in detecting the early vascular changes in brain uptake in early diabetes.

**Materials and Methods**

**Materials:** HMPAO (Exametazime) kit was purchased from Amersham (UK). $^{99m}$Tc was eluted from a fresh $^{99}$Mo-$^{99m}$Tc generator (Amersham, UK). All chemical materials used in this study were supplied by Sigma-Aldrich (UK) unless otherwise stated.

**Animals:** In this study, male Wistar rats (n=12) of 200 g weight were handled in accordance with the ethical standards, which was approved by the local ethics committee and recommended by the Helsinki Declaration.

**Preparation of $^{99m}$Tc-HMPAO:** Fresh elutes of technetium ($^{99m}$Tc) were obtained on each time to prepare the $^{99m}$Tc-HMPAO following the manufacture’s instructions and recommendation. The preparation of $^{99m}$Tc-HMPAO is simple, by adding 1110-2960 MBq of $^{99m}$TeO$_4$ in 5 ml of saline to a freeze-dried Exametazime kit.
**Induction of Experimental Type 1 Diabetes:** Experimental type 1 diabetes was induced in rats by intraperitoneal (i.p.) injection of 55 mg/Kg streptozotocin (STZ) dissolved in citrate buffer. Control rats were injected with the buffer. Therefore, two groups were used in this study: control group and diabetic-STZ group and each group contained 6 rats.

**Experimental Protocol:** At the time of an experiment, rats were taken randomly, numbered, and weighed. In order to not experience pain or disturbance, intravenous line was placed in advance before the radiopharmaceutical injection. The intravenous line was placed in the dorsal vein of the tail of the rat. Each rat was anaesthetized by intraperitoneal injection with 0.5 ml of 0.5 g intraval sodium for 5 min before $^{99m}$Tc-HMPAO injection. This amount of anesthesia was enough to continue till the later imaging time points of 2 and 4 h. Another intraperitoneal injection of 0.5 ml of 0.5 g intraval sodium 5 min before imaging was administered for 24 h time point imaging. This type of anesthesia was affecting neither the blood pressure nor the biodistribution of the radiopharmaceutical in the brain and background region. An amount of 1 ml of 129.5 MBq of $^{99m}$Tc-HMPAO was injected within 30 min of $^{99m}$Tc-HMPAO preparation and saline was used to push the activity via the fixed intravenous line. Each rat from each group was undergoing brain scan at 30 min after $^{99m}$Tc-HMPAO injection.
**Gamma Camera Imaging:** Brain scan was performed using a single-head gamma (γ) camera (Philips camera; Odyssey LX) equipped with a high-resolution parallel hole collimator connected to Dell computer. The matrix was 128×128 pixels and the photopeak was focused at 140 KeV with a symmetric 10% window. Planar (posterior static views) images were obtained for 5 min acquisition time at each time point. Each anaesthetized rat was fixed with plastic tape on a fixing board against the table of the γ camera during acquisition time. The rat’s head was localized in the center of the field of view and zoom factor of 4 was applied during each acquisition time.

**Image Processing:** Data processing for brain scan was done by drawing a region of interest (ROI) circulating the brain (B) and a ROI around the soft tissues around the neck of each rat as background (BKG). Brain $^{99m}$Tc-HMPAO uptake minus background (net brain counts; NBC) in control rats were determined first and expressed as the mean ± standard deviation (mean±SD). Then NBC in diabetic-STZ rats were studied.

**Data Presentation and Statistical Analysis:** All data - unless otherwise stated- was expressed as the mean ± standard deviation of the mean (mean±SD). Statistical significance (P) of the results was determined by the Student's paired $t$-test. A P value of less than 0.05 was taken as the minimum level of significant difference. All statistical analysis was performed using the "SPSS 13.0 version" software.
Results

The net $^{99m}$Tc-HMPAO brain counts (NBC) in control group (mean±SD) was: $67766.83±10405.19$, $49439.67±6960.06$, $37080.83±5459.16$, $2017.00±302.72$ counts per sec (cps) at 0.5, 2, 4 and 24 h respectively, whilst NBC in STZ group was: $241296.83±3548.81$, $148215.17±2316.45$, $111254.17±1682.65$, $6817.83±76.36$ cps at 0.5, 2, 4 and 24 h respectively. A statistical comparison (Student's $t$-test) between the NBC controls and the NBC diabetic-STZ group at each time point was determined. There was highly significant difference between control and STZ rats at each time point ($P < 0.0001$).

Figure 1 shows $^{99m}$Tc-HMPAO net brain counts (NBC) versus imaging time points: 0.5, 2, 4 and 24 h for control and diabetic-STZ group.

Discussion

The diagnosis of the brain disorders with $^{99m}$Tc-HMPAO is a very important imaging tool in the nuclear medicine department. In this study, there was a highly significant change in the uptake of $^{99m}$Tc-HMPAO between the control and diabetic-STZ group.

Our preliminary results of this study showed increased $^{99m}$Tc-HMPAO brain uptake in the early one week-duration diabetes in the diabetic-STZ group compared to control group along all time points from 0.5 h to 24 h. This is may be due to early pathophysiological changes such as
vasodilatation in the early diabetes. But this was not in agreement with one study in human patients with type 2 diabetes that showed 25 to 30% reduction in regional cerebral blood flow compared to control subjects [7].

Usually blood flow parallels rate of glucose metabolism in the brain tissue. The increased $^{99m}$Tc-HMPAO brain uptake in this study may be related to increased rat brain demand for/or accumulation of glucose uptake in early diabetes. $^{99m}$Tc-HMPAO uptake was reported to accumulate in human (MCF-7) breast tumor cell lines in vitro [9]. This accumulation was significant and related to enhanced rate of glucose metabolism of these malignant cancer cells, which facilitated their detection using $^{99m}$Tc-HMPAO. However, a study with type 1 diabetes in patients reported a 15 to 20% reduction in cerebral glucose metabolism using PET, but only in a subgroup of patients with long-standing diabetes and there were no abnormalities in glucose metabolism observed in patients with newly diagnosed diabetes [10].

Conclusions

$^{99m}$Tc-HMPAO brain scan has an important role in the diagnosis of early diabetes as early as one week duration in a rat animal.
References


Figure 1: $^{99m}$Tc-HMPAO net brain counts (NBC; cps) versus imaging time points: 0.5, 2, 4 and 24 h for control (mean±SD): $\circ$ Mean$_C$, open circles and diabetic-STZ (mean±SD) group: $\bullet$ Mean$_{STZ}$, black circles.
Figure 1

Net Brain Counts (NBC) vs Time

99m-Tc_HMPAO (cps)

Mean_C

Mean_STZ

Time (h)