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The sensitivity and specificity of ^{99m}Tc-IgG radiotracer to differentiate infection lesions induced by Staphylococcus aureus and sterile inflammation lesions induced by carrageenan assay in rat's foot

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Abstract

Background: Detection and identification of infection from sterile inflammation foci has a crucial role in diagnosis and therapy of patients in clinical practice.

Objective: To assess the efficiency of labeled human polyclonal immunoglobulin with technetium 99m in order to detect septic or aseptic lesions which were induced in a rat model.

Methods: The freeze-dried IgG kits have been reconstituted by ^{99m}Tc. The radio conjugate yield, radiochemical impurities and stability radio complex were performed by ITLC (Instant Thin Layer Chromatography) and Gel filtration assays. Twenty adult, male NMRI (Naval Medical Research Institute) rats were randomly divided into two groups equally. Infection was induced by Staphylococcus aureus and sterile inflammation created by Carrageenan test. All lesions were created in the rat's foot. Then radioisotope investigations were undertaken.

Results: Labeling yield was approximately 98%. The radio complex showed good stability in normal saline. All affected feet could be easily visualized by imaging in qualitative study. The value of target to non-target ratio at the infection (n=10) and sterile inflammation (n=10) were 2.81±0.16 and 1.54±0.15 with $p<0.007$. Therefore, the radiotracer uptake at the septic lesions was significantly higher than the aseptic lesions.

Conclusion: Imaging with ^{99m}Tc-IgG is highly sensitive to localized infection or inflammation foci. The increased accumulation of radiotracer at the infection versus inflammation foci may be helpful to interpret the image.

Keywords: Carrageenan, Infection, Inflammation, ^{99m}Tc, ^{99m}Tc-IgG

1. Introduction

The discrimination of infection from sterile inflammation is still one of the most critical situations in clinical practice, because it plays an important role to choose the appropriate treatment of patients, especially in preventing the loss of golden time. The imaging techniques, along with other medical measures, have an effective role in differential diagnosis between infections from sterile inflammation lesions. The available imaging techniques such as plain radiography, ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) are assessed in the structural changes in tissues or organs. They are sensitive but not specific for distinct infection or

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sterile inflammation, particularly in early phase of disorder when anatomic structure of tissues or organs has not been changed (1, 2). Nuclear medicine can be used in the protocol to determine septic from aseptic lesions (3). In this method, the physiological changes in organs are evaluated, therefore, it potentially has an effective role in the correct diagnosis of infection or sterile inflammation, along with other clinical findings. A variety of different radiopharmaceuticals were examined in this regard. Each radiopharmaceutical has special advantage and disadvantage characteristics. None of the available radiopharmaceutical agents that are used for the diagnosis of infectious or inflammation disorder in nuclear medicine are ideally suited from the aspect of biodistribution, pharmacokinetics or selective accumulation at the septic or aseptic foci. Therefore, various research centers are trying to develop a radiotracer that can meet these criteria. The label polyclonal antibody with Indium111 (^{111}In) or technetium-99m (^{99m}Tc) is one of the promising radiotracers for imaging detection of infection and inflammation lesions (4, 5). The Indium-111 radioisotope is not an ideal radionuclide in nuclear medicine departments. It has inappropriate long physical half-life, multiple energy gamma radiation and from the aspect of dosimetry is unfavorable because of the high total body absorbed dose (6). In addition to the above-mentioned factors, ^{111}In is the product of cyclotron and relatively highly expensive, and is not available as a generator. The ^{99m}Tc radioisotope is widely used in diagnostic procedures in clinical practice. Its popularity is mainly due to the fact that the radioisotope can be readily produced by ^{99}Mo/^{99m}Tc generators. Therefore, ^{99m}Tc-IgG can be demonstrated as an ideal radiotracer for radioisotope imaging. According to the literature, the infection or inflammation foci could be detected by ^{99m}Tc-IgG scintigraphy imaging (7-10). It indicated that ^{99m}Tc-IgG scintigraphy imaging is highly sensitive in detecting septic or aseptic lesions. But the selectivity of ^{99m}Tc-IgG scintigraphy imaging has not been clearly elucidated till now. It is completely justified to evaluate the specificity of ^{99m}Tc-IgG radiotracer to detect infection or inflammation by the valid and reliable experimental method. Carrageenan test is used for the creation of sterile inflammation in order to evaluate the anti-inflammatory effect of drugs or any other compounds in animals (11, 12). This investigation was launched to analyze the susceptibility and selectivity of ^{99m}Tc-IgG radiotracer to distinguish septic and aseptic lesions induced by Staphylococcus aureus (S aureus or Carrageenan respectively in rat's foot).

2. Material and Methods

2.1. Setting and design

The practical research work was carried out from May 2016 to October 2016. The chemical and solvents reagents with analytical grade were procured from Merck and Sigma-Aldrich companies and used without further purification. The freeze-dried IgG kits and ^{99}Mo/^{99m}Tc generators were supplied by the Radioisotope Division of the 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 % sterile and apyrogenic saline as elution solution. The rats, with average weight of 140±20 g, were used in animal study. The subjects that participated in this approach were supplied by the research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences.

2.2. Bacteria Sample

The culture of the Staphylococcus aureus sample was performed according to the procedure that has been previously reported (13, 14). Briefly, suspected S aureus samples were collected and transferred to the laboratory from the patients admitted to the infection ward of Razi General Public Hospital in Ahvaz, Khuzestan Iran. The isolates were inoculated on blood agar culture media and incubated for 24 hours at 35 ºC aerobically. From the aspect of morphology, the gram-positive cocci bacteria in pairs or clusters were selected. The catalase, coagulase, mannitol fermentation and DNase tests were undertaken in order to identify S aureus strain. Finally, S aureus strain was cultured in the Muller-Hinton agar culture media.

2.3. Animal Study

This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences with the code IR.AJUMS.REC.1395-36. In this investigation, all the guidelines specified in the Ahvaz University of Medical Sciences were followed in order to conduct research on animals. Therefore, a total number twenty adult male NMRI were selected for this research work. For in-vivo study, the rats were exposed to the lab condition for a period of one week. The subjects were kept in separate cages in an air-conditioned room at 24±1 °C with a 12 hours light-dark cycle. The feeding of rats was carried out by standard pellet and, allowed free access to water during this investigation. The rats were randomly categorized into two equal groups. All lesions were created on the foot of the animals.

2.4. Induced infection lesion

The rats were briefly anesthetized by diethyl ether. Half milliliter saline containing 10^{10} CFU of viable S aureus was injected into the rat's foot. The injected area was washed with normal saline and then the subjects were transferred to their cages. Radioisotope investigation was performed 48 hours after inoculation of bacteria, when swelling was visible at the infection site. Animals were fed with standard pellets and had access to water during this period.

2.5. Induced sterile inflammation lesion

The sterile inflammation lesion was induced by injection of half milliliter of 3% Carrageenan solution in normal saline on the experiment day. All lesions were created in the right thigh muscle of the rats. The subjects were under brief anesthesia by diethyl ether in order to relieve the pain of Carrageenan effects. The injected area was irrigated with normal saline. The sterile inflammation with visible redness and swelling was developed 2 hours post injection. Maximum effect of Carrageenan was observed between 2 to 4 hours post injections and persisted for more than 12 hours.

2.6. Preparation ^{99m}Tc-IgG and quality control

The freeze-dried IgG kits (AEOI, Tehran, Iran) were used and labeling of IgG with ^{99m}Tc was performed as follow: The lyophilized IgG kits were taken from the freezer and put at room temperature. After reaching the ambient temperature, 1 ml of normal saline was incubated with a 2 ml syringe. It was entered into the vial after adjustment to the size of the syringe. The vial should contain a vacuum and the solution should be pulled in by itself. Then 740 MBq (20mCi) freshly eluted $Na^{99m}TcO_4$ in 1 ml of saline was added to the vial and incubated for 20 min at room temperature in a lead shield container. Instant Thin Layer Chromatography (ITLC) and Gel Filtration Chromatography analysis were performed in order to quantify the yield and for stability of radio conjugate radiotracer. The silica gel 60 (Merck) filter paper chromatography was used as the stationary or the solid phase and two different mobile systems were used in the ITLC study. When normal saline was used as the mobile phase system, The R_f values were as follow: $^{99m}TcO_2$ and ^{99m}Tc-IgG=0.0; ^{99m}Tc-MDP and free $^{99m}TcO_4^-$ = 0.9–1.0. Whereas the solvent mixture of NH3/H2O/ethanol in a ratio (1:5:2) was used as another mobile phase system, the R_f values were $^{99m}TcO_2$ =0.0; ^{99m}Tc-IgG, ^{99m}Tc-MDP and free $^{99m}TcO_4^-$ = 0.9–1.0 respectively. Each strip was cut to ⅓ lower and ⅔ upper parts, and activity of each part was recorded for 2 minutes by gamma camera equipped with low energy all-propose collimator using an energy peak centered a140keV with NaI (Tl) detector (Aktivimeter, Siemens, Germany). The stability assessment was also checked in PD10 column (Sephadex G-25) each time. Therefore, 100 µl of labelled antibody was placed on a PD10 column. After that, for different time point, the column was washed with 30 ml PBS solution. The volume of 1 ml fractions of eluted solution was accumulated in separated test tubes and measured in a well-type gamma counter, which was specified.

2.7. Radioisotope analysis

The rats were placed in the restrainer apparatus and the 37 MBq (1mCi) ^{99m}Tc-IgG was intravenously injected through the tail vein into each subject. The injected area was rinsed with normal saline and the animals were transferred to their cages. Each rat was kept separately during this period. Radioisotope analysis was carried out 1 hour post injection. For imaging, it was necessary for each rat to be anesthetized with diethyl ether, then they were fixed on a board by surgical tape, while each subject was laid back with limbs spread out. Scintigraphy was performed with a single-headed camera (E-Cam, Siemens USA) equipped with a low-energy high resolution collimation in all images. Acquisition parameters were as follow: matrix size 256×256, Zoom factor ×3, anterior and posterior views for 5 min and energy window 140 Kev and filter back projection was used for reconstitution. Whole body scan and anterior and posterior static images was acquired using a large field of view gamma camera peaked 140 Kev with a 15 % window and a low energy all-purpose collimator for 500 kilo counts per image. The position of the gamma camera was the vicinity of the affected foot and contralateral healthy foot in order to investigate the radiotracer uptake at the created lesions by infection or sterile inflammation. Therefore, by using commercial software, the region of interest (ROI) areas were drawn over the affected foot as target and contralateral foot as non-target. The ratio of target to non-target was obtained by dividing count per pixel in the affected foot to count per pixel in the unaffected foot. The back ground subtraction was not used in our calculation in all studies. The interpretation of images and the measurement of target to non-target were undertaken by a nuclear medicine physician. The interpreter did not know the nature of the induced injury on the rat's foot. Due to the fact that it was not possible to directly measure the activity of other organs of the animal for the superimposition activities, the rats were sacrificed by diethyl ether after imaging. The organs such as affected foot, unaffected foot, kidneys, liver, stomach, spleen, intestine, bladder, heart and lungs were removed and weighed. The activity of each organ was

counted by gamma counter. The relative activity of each organ was calculated by dividing the activity of organ into the total activity of isolated organs from the animal. The results obtained from this analysis are stated in Table 1.

2.8. Statistical analysis

Microsoft Excel software was used to calculate the means and standard deviations and the data were expressed as the mean ± SD. Repeated measure design analysis of variance followed by Tukey test was used to assess the difference between the accumulations of ^{99m}Tc-IgG radio complex at the septic and aseptic foci. Any p value less than 0.05 was considered to be statistically significant.

Table 1. Biodistribution of ^{99m}Tc-IgG in rat*

Relative uptake	Organ									
	Liver	Kidneys	Intestine	Affected foot	Unaffected foot	Stomach	Lungs	Spleen	Bladder	Heart
Infection	34.09±3.2	24.24±3.15	14.21±1.21	12.5±0.88	4.57±1.1	2.81±0.36	2.63±0.7	1.52±0.38	2.2±0.65	1.22±0.36
Inflammation	40.73±4.21	21.18±2.01	12.84±1.44	7.08±0.65	4.85±0.5	4.76±0.35	3.45±1.6	2.1±0.32	1.9±0.51	1.07±0.15

*Quantitative analysis was performed after radioisotope imaging. Therefore, the rats were killed by diethyl ether, and organs of interest such as liver, kidneys, intestine, affected foot, unaffected foot, stomach, lungs, spleen, bladder and heart were removed. The relative activity of each organ to the interest organs was calculated. The data in the top row belong to the radiotracer biodistribution in the rats (n=10) infected with S aureus. The data in bottom row belong to the radiotracer biodistribution in the rats (n=10) with sterile inflammation lesion, which were induced by Carrageenan assay

3. Results

The freeze-dried IgG kit contained the ligand of methylene diphosphonate (MDP) for ligand exchange in order to produce ^{99m}Tc-IgG radiotracer with appropriate yield. The ITLC assay indicated that the radio complex yield was approximately 98% 1 hour after labeling. The stability of labeled antibody was performed by gel filtration analysis. The early fraction was related to label antibody while the second and third fractions were related to free $^{99m}TcO_4$ and ^{99m}Tc-MDP. The remained activity in the column was related to $^{99m}TcO_2$ colloid. The yields of desired radiotracer (^{99m}Tc-IgG) and radio impurities ($^{99m}TcO_4$, ^{99m}Tc-MDP and $^{99m}TcO_2$) were approximately 99.1, 0.22, 0.18 and 0.5 % 1h post reconstitution, respectively. The labeling yield decreased to 82.5% for ^{99m}Tc-IgG but radiochemical impurities increased ^{99m}TcO 4.4%, ^{99m}Tc-MDP 3.55% and $^{99m}TcO_2$ 10% after 24 hours post labeling. These analyses demonstrated that the ^{99m}Tc-IgG radio conjugate was prepared with suitable yield and showed a good stability in normal saline. To prevent any misinterpretation of images, all lesions were created on the right foot of rats in this study. The visual evaluation of images indicated that the images had appropriate quality in each case. The quality of images did not change over time, so it was possible to calculate the target to non-target ratio directly. All affected regions could be visualised by scintigraphy imaging. This matter indicated that the radiotracer uptake at the septic or aseptic lesions had been carried out to the extent that these regions could be identified in the scintigraphy imaging. Therefore, the scintigraphy with ^{99m}Tc-IgG was sensitive that all lesions were readily detected by imaging. As it is stated in Figure 1, the radiotracer uptake at the septic site is higher than the aseptic site. The color scale bar was used for the changing color scale and to increase or decrease picture contrast. The contrast depends on the amount of gamma photons emitted from the tissue and it directly correlates with the radiotracer uptake on the region of interest. It should be increased to better show the region on interest particularly in the regions with low counts compared with areas with normally high uptake such as the liver. The infection versus the sterile inflammation lesion was visualized with lower contrast. It revealed that the accumulation of radiotracer at the septic site is higher than the aseptic site. The ratio of target to non-target values for septic (n=10) and aseptic lesions (n=10) were 2.81± 0.16 and 1.54±0.15 respectively. Therefore, the radio conjugate accumulations at the septic lesions with $p<0.007$ were significantly higher than the aseptic lesions. It revealed that the relative selectivity toward septic versus aseptic foci could be observed by ^{99m}Tc-IgG scintigraphy scanning. The measurement of relative activity of other organs has been performed in order to provide further information about the biodistribution of ^{99m}Tc-IgG radio complex in rats. As it is shown in Table 1, the pathologic condition-induced infection or sterile inflammation could not lead to a significant difference of organ distribution, except the radiotracer uptake has been enhanced at the infection in comparison to inflammation foci. The mean ratio of affected foot to unaffected foot was calculated for both groups of animals. These values for septic and aseptic lesions were 2.74 and 1.46 respectively (Table 1). It indicated that the radiotracer uptake at septic foci was higher than aseptic foci. This achievement was very consistent with the target to non-target ratio. The highest radioactivity was measured in the liver followed by kidney and intestine. It revealed that the metabolism of radio conjugate samples was carried out by the liver. The biodistribution pattern of radiopharmaceutical was similar in the two groups of animals.

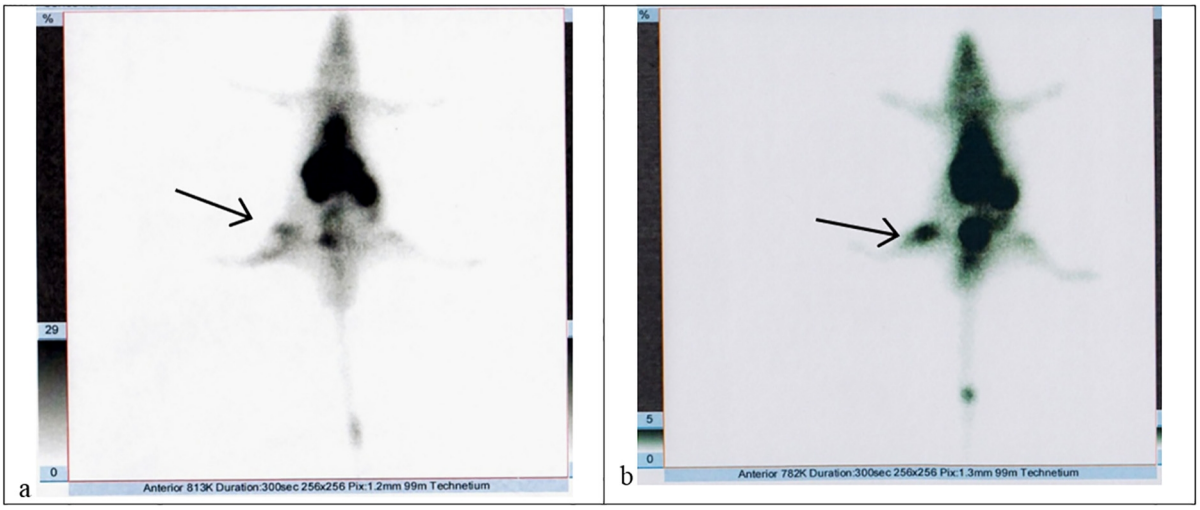

Figure 1. Scintigraphy Imaging. ^{99m}Tc-IgG radioisotope imaging has been performed 1 hour after 37 MBq (1 mCi) radiotracer injected by contra lateral tail vein. The anterior view images demonstrate lesions a: infection induced by Staphylococcus aureus, and b: sterile inflammation induced by Carrageenan assay.

4. Discussion

The most challenging step in the management and treatment of disease is the accurate diagnosis in every clinical practice. The potential risk of inappropriate treatment could lead to the progression of disorder and loss of golden time to treat illness, and ultimately increase the number of admissions to hospitals. The identification of infection from sterile inflammation is one of the most important issues in nuclear medicine. The potential capability to detect infection specifically, is a very useful method in the management of patients, particularly suffering from fever of unknown origin and in such other suspected conditions of occult infection. Radioisotope imaging has a crucial role in the identification of infection from sterile infection lesions. Therefore, different radiopharmaceutical agents have been established or examined as potential detecting agents for infection (15-18) or sterile inflammation (19, 20) in both preclinical and clinical investigations. An ideal radiopharmaceutical agent has the following characteristics to distinguish infection or inflammation lesions. It has high sensitivity to the infection or inflammation site. It should discriminate infection from inflammation. The toxicity and immunogenic reactions have not been observed after administration of radiotracer agent to the patient. It has a rapid clearance from the blood and no has gastrointestinal uptake. In addition to the aforementioned factors, the reconstitution of cold kit is straightforward and affordable at the nuclear medicine departments. Since currently available radiopharmaceutical agents is not ideal in this regard, different research efforts are made to developed an agent that meets these ideal requirements (21-23). ^{67}Ga-Citrate is the most primitive radioisotope for the detection of infection from sterile inflammation. The scintigraphy imaging with this radioisotope is hindered by the following precaution factors. It has long physical half-life, high and multiple energy gamma radiation causing high radiation absorbed doses and high sensitivity for both infection and non-infection inflammation. In addition to the above-mentioned factors, ^{67}Ga radionuclide is the product of cyclotron and is relatively expensive and is not available as a generator (24). Three-phase scintigraphy imaging with ^{99m}Tc-MDP radiotracer is highly sensitive, particularly when the radiotracer uptake is positive in all three phases. Imaging is provided to differentiate soft tissue from bony infection. In spite of high sensitivity three-phase bone scan, it is not specific for tagged infection sites. The radiotracer uptake can be enhanced non-specifically due to the presence of inflammation (25). Leukocytes labelled with ^{111}In and ^{99m}Tc radioisotopes have been considered as a gold standard in order to discriminate septic from aseptic lesion. It is necessary to take blood from the patient in order to label leukocytes. Then the separation of leukocytes must occur and labelled with radionuclide and in the last step, the labeled leukocytes are injected to the patient in the aseptic condition. The radiolabeling process is time-consuming and has potential inherent risk of contamination or transmission of blood-borne pathogens to patient or technician. In addition to the above-mentioned factors, this method requires specialized facilities and must be carried out by expert staff in this regard. Moreover, the labelled leukocytes radiotracer cannot be used in neutropenic subjects (26, 27). The broad spectrum antibiotic molecules have been recommended as alternative radiopharmaceutical agents to detect infection. These molecules can be accumulated and metabolized by microorganisms. Sterile abscesses are not detected by radiotracer uptake, because the labelled antibiotic cannot be bound to dead bacteria. Several broad antibiotic agents have been evaluated in this field. The majority of antibiotic

molecules have been examined for radiolabeling are fluoroquinolones, and second and third generation cephalosporin (28, 29). Labelled ciprofloxacin with ^{99m}Tc radionuclide, known as Infecton, has been developed as an infection-seeking radiotracer among the antibiotic agents. Infecton is a combination of the benefits of using a broad spectrum antibiotic that can be attached to bacterial DNA gyrase and radiolabeling with ^{99m}Tc radionuclide. According to the literature, Infecton could demonstrate acceptable and promising sensitivity in detecting a wide variety of septic lesions (30-33), but it could not differentiate between infection and sterile inflammation foci (13, 34). Radioisotope scintigraphy imaging with Infecton is impeded by the other disadvantage factor. Pathogenic microorganisms have been resistant to the antibacterial effect of ciprofloxacin due to its high consumption, misuse and inappropriate prescriptions (35, 36). Furthermore, ^{99m}Tc-Ciprofloxacin scintigraphy imaging does not have any value to visualize and localize infection lesions if microorganism pathogens are resistant to ciprofloxacin (37). Several radiopharmaceutical agents have been assessed for the diagnosis of infection and sterile inflammation (38, 39). However, no radiotracers have been studied until now that fulfills all the characteristic parameters of desire an ideal, each has its own advantages and disadvantages. Infection is the result of invasion, proliferation and presence of microorganism pathogens in the body, while inflammation is the natural response of the immune system against any other type of disorder or injury. Therefore, infection without inflammation or inflammation without infection may occur. It is contingent upon the cause of the injury or disease. Carrageenan is a natural polysaccharide compound and is obtained from edible red seaweeds. It can cause sterile inflammation without damage to the inflamed tissue after administration to animals. The inflammatory reaction induced by Carrageenan is similar to that of the natural inflammatory process (12). Various molecular mechanisms have been proposed for induction of sterile inflammation by Carrageenan. A variety different inflammatory mediators are produced and released sequentially at the site of inflammation lesion following the injection of Carrageenan. Histamine, bradykinin and serotonin are released in the early phase of inflammation. The permeability of blood vessels is enhanced by prostaglandins in the process of inflammation. Other pro-inflammatory mediators such tumor Necrozing factor (TNF), interleukin 1 and 6 (IL-1 and IL-6) play a pivotal role in inducing local inflammation. In addition to the above-mentioned mechanisms, local neutrophil activation and infiltration are involved in the inflammatory responses (40-42). Human polyclonal immunoglobulin IgG is a non antigen specific antibody. IgG can be labelled with ^{111}In and ^{99m}Tc radionuclides. It does not produce allergic reaction response because its source is of human origin. The superiority of ^{99m}Tc-IgG to ^{111}In-IgG is related to the labeling process. The most intensively used radioisotopes is ^{99m}Tc in nuclear medicine. Its popularity is related partly to its ideal radioisotope and chemical characteristics for radiolabeling of different ligands that have unbounding electrons such as hydroxyl, nitrogen, sulfur, and phosphorus etc. It has the ideal gamma energy (140 keV) which is suitable for gamma camera detection and appropriate half-life ($t_{½}$=6 h). In addition to the above-mentioned factors, it is available as a generator for radiopharmaceutical works. IgG can be readily labeled with ^{111}In for radioisotope imaging at the time points beyond 24 hours. The accumulation of labeled IgG at the injured area occurs by non-specific manner. Localization of radio labeled IgG is mainly attributable to the increased vascularization, extracellular volume, and endothelial permeability with the inflamed region. The inflamed area was created at the septic and aseptic foci. S aureus were present at the infection site and radio conjugate could interact with bacteria in addition to the above mentioned factors that radiotracer was accumulated at the inflamed region. Therefore, the uptake of radiotracer at septic sites was higher than the sterile inflammation foci. The radiotracer was readily accumulated in inflammatory regions to an extent sufficient enough to yield the images with appropriate quality. For this reason, the injured areas could be effectively identified by visual inspection of images. The relative specificity could be considered by target to non-target ratio and quantitative assays in this study. It is required to launch the clinical investigation in order to quantify the ^{99m}Tc-IgG radiotracer uptake at infection and inflammation lesions. When these data indicate that the statistical difference of radiotracer uptake is observed between infection and inflammation sites, it can be considered as standard criteria for diagnosis of infection and inflammation by ^{99m}Tc-IgG scintigraphy imaging. This approach was one of the first studies performed, to investigate the sensitivity and specificity of ^{99m}Tc-IgG radioisotope imaging to distinguish infection and sterile inflammation foci by using a reliable experimental animal model.

5. Conclusions

Carrageenan assay can be considered as a reliable test for the evaluation of sensitivity and selectivity of any radiopharmaceutical, and has been examined as an infection-seeking agent in a preclinical investigation. The outcome of the investigation indicated that ^{99m}Tc-IgG radioisotope imaging is highly sensitive in detecting infection or inflammation foci by visual inspection of images. Other medical trials must be considered for intelligent interpretation of ^{99m}Tc-IgG scintigraphy imaging. The radiotracer uptake at the affected foot as target versus unaffected foot as non-target is one of these modalities. This factor may be helpful in distinguishing infection from sterile inflammation lesions.

Abbreviations:
Bq: Becquerel, Ci: Curie, Mo: Molybdenum, TcO_4^-: Pertechnetate, Tc: Technetium

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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