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Assessment of Metastatic Status of Axillary Lymph Nodes using Intravenous Fluorescein in Carcinoma Breast: A Novel Approach in Surgical Management

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ABSTRACT

Background: Assessment of lymph node metastasis is of prime significance for staging and treatment planning for breast cancer. All enlarged lymph nodes do not contain metastasis. Sentinel lymph nodes biopsy is recommended for patients with clinically negative axilla. But there are no standard guidelines for managing patients with enlarge axillary lymph node. In the present paper we have evaluated the role of intravenous Fluorescein to identify the axillary nodal metastasis during axillary dissection.

Aim: Identification of metastatic fluorescent node during axillary dissection.

Methodology: The present cross-sectional study at All India Institute of medical sciences on 32 operable carcinoma breast cases were enrolled in the study. Fluorescent and non-fluorescent nodes were sent separately for histopathological evaluation.

Result: Present study include a total of 32 cases. From these 32 cases a total of 267 lymph nodes were separated out in which 185 nodes were fluorescent and 82 nodes were non fluorescent. Out of 267 fluorescent node 103 were histologically positive and remaining nodes were free of tumor. In non-fluorescent nodes only 6 nodes show the presence of tumor.

Conclusion: Intravenous 20% fluorescein sodium has a high sensitivity of 94.5% & specificity of 48% with positive predictive value of 55.7%, which is comparable to conventional sentinel lymph node procedure in detecting early metastasis. Besides time saving and non-toxic, this technique predicts the metastatic status of axillary lymph node during surgery, aiding appropriate surgical intervention.

Keywords: Fluorescence imaging, Breast cancer, Sodium Fluorescein, Axillary lymph node.

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1 Introduction

Cancer is a leading cause of death worldwide that accounts for nearly 1 in every 6 deaths. In 2020, there were an estimated 19.3 million new cancer cases and nearly 10 million cancer deaths globally [1]. By 2025, the global burden of new cancer cases is expected to reach 21.9 million and by 2040, it is projected to 30.2 million new cancer cases [2].

Breast cancer was one of the most commonly diagnosed new cancer cases globally in 2020 [1]. Female breast cancer occurs in every country of the world at any age after puberty but with increasing rates in later life. As of the end of 2020, approx. 7.8 million women were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer [3]. Globally, incidence of breast cancer in women has been increasing that surpassed lung cancer as the most commonly diagnosed cancer. An estimated 2,261,419 new cases were diagnosed in women across the world in 2020 [2].

By 2025, the global burden of new breast cancer cases is expected to reach 2.5 million and by 2040, it is projected to 3.19 million new breast cancer cases[2]. In India alone the estimated number of new breast cancer cases were nearly 178k in 2020 which is projected to reach 201k in 2025 and 272k new breast cancer cases by 2040 [2].

In India breast cancer trends has been increasing trend towards younger age group (below 40 years) [4] and aggressive in the younger age group [5]. Breast cancer is dominated by lymphatic metastasis and many patients presented with advance stage having possibility of axillary lymph node (ALN) metastasis. Hence it is important to evaluate the exact metastatic status of axillary node (AN) in these patients. Axillary lymph node dissection (ALND) is the standard care of treatment for the node positive breast cancer patients but it is associated with significant morbidity in terms of lymphedema, seroma, pain parasthesias and shoulder stiffness [6]. Sentinel lymph node (SLN) has the highest probability being affected as it is the first lymph node (LN) to receive lymphatic drainage from the breast. If the SLN does not been metastasized, the probability of the remaining axillary lymph node (ALN) metastasis is quite low [7].

Sentinel lymph node biopsy (SLNB) is the traditional method to assess metastatic status of ALN for breast cancer patients. This method is less invasive and cost effective. Axillary lymph node dissection (ALND) can be spare for the SLNB negative patients [8], [9]. But false negative is the critical problem which leads to local recurrence and low prognosis [10]. Therefore, accuracy in the SLNB is needed for better prognosis. In this procedure a vital blue dye or radioactive isotope or both is injected, intradermally near the tumor [11]–[14]. Dye guided method is safe and convenient compared to radioisotope method which require adequate training, costly isotope facility [15]. Dye-stained axillary lymph nodes are assessed for radioactivity using gamma probe and sent for frozen section examination. This procedure has 5 to10 % false negative rate [16]–[19]. This high false negative rate creates a fear of recurrence of disease in the mind of surgeon. Hence there is a need to explore a quick & reliable method of detecting cancer in axillary lymph node.

1.1 Proposed Method

We propose a quick method for the identification of metastatic status of axillary lymph nodes during axillary dissection using Intravenous Fluorescein in breast cancer patients.

1.2 Proposed Hypothesis

Tumor growth is dependent on angiogenesis [20]. When tumor achieves a diameter more than 2 mm tumor cells begin to secrete "Vascular endothelial growth factors" resulting in increased angiogenesis. This neovascular network of blood vessels increase the blood flow in the lymph node containing metastasis. The increased blood flow in these lymph nodes allow higher concentration of intravenously injected fluorescein. Fluorescein containing lymph node can be detected by fluorescence when viewed with blue light during sentinel node biopsy.

2 Material and Methods

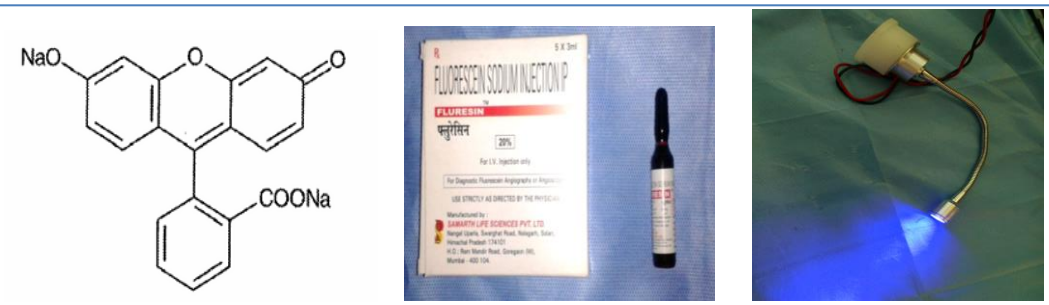
This is a prospective research study conducted in the department of surgery, All India Institute of Medical Sciences, New Delhi, India. In this study thirty-two biopsy proven cases of carcinoma breast were included after informed written consent. The project was approved from institute ethical committee. In the present study, 2 ml of sodium fluorescein (20% solution) was injected intravenously just five minutes prior to the surgery. After incision the fluorescent LNs were detected using blue light.

2.1 Patient Criteria

Operable carcinoma breast cases above 18 years of age with no palpable node in axilla (Clinically tumors with T1 or T2 status by TNM classification with no metastasis or no palpable axillary or supraclavicular lymph node enlargement) were included in the study. Cases with distant metastasis, lactating, pregnant, male patients, and patients with palpable and sonography proven axillary lymph nodes were excluded from the study. Detailed clinical parameters of the patients (Age, Size of tumor, tumor type) were noted.

2.2 Surgical Procedure

After inducing anesthesia, a dose of 2 ml sodium fluorescein (20% solution) was injected intravenously, just 5 min prior to the surgery. Molecular structure of fluorescein sodium and packed ampule is shown in figure 1. After performing axillary incision, a blue light of wavelength 460 nm (shown in figure 1c) was used to excite the fluorescein that emits greenish yellow light of wavelength 520 nm. Fluorescent nodes were examined in vivo as wells as on the resected specimen & images of tissue were captured. Fluorescent and non-fluorescent nodes were sent separately for frozen section evaluation, based on which decision of the extent of surgery (ALND sparing or not) was taken.



(a) (b) (c)

Figure 1: (a) Molecular structure of fluorescein sodium, (b) packed ampule, (c) Blue light having wavelength 460 nm

2.3 Histopathological Examination

The fluorescent and non-fluorescent nodes from the resected specimen were separated and sent for histopathological examination. Histopathologist reported fluorescent and non-fluorescent nodes separately along with the main specimen. Post-surgery data on fluorescent and non-fluorescent nodes were compared with histopathological data to check the sensitivity of the proposed method.

3 Results

Out of 32 cases a total of 267 nodes were separated out (185 were Fluorescent and 82 were non- fluorescent). Fluorescent nodes can be seen from figure 2. Among 185 fluorescent nodes 103 nodes were positive on histopathological examination (HPE) & remaining 82 were negative on HPE. Among 82 non-fluorescent nodes (can be seen from figure 3) only 6 nodes showed tumor positivity while 76 were free of tumors.

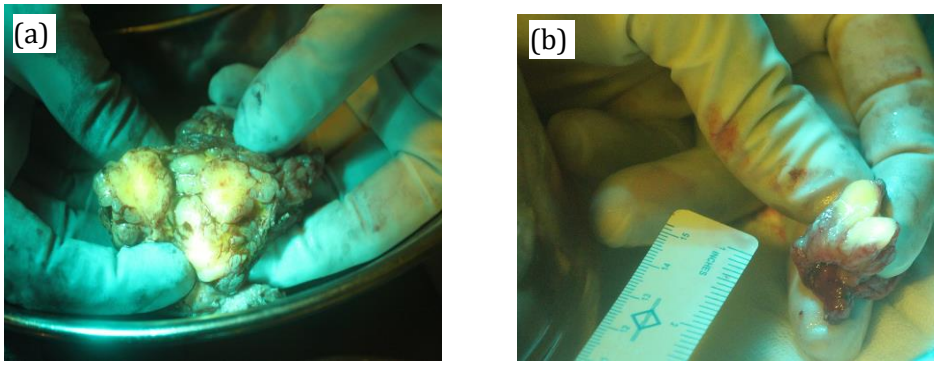


Figure 2: (a) Matted Fluorescence axillary nodes, (b) Single axillary fluorescence node.

Figure 3: Non fluorescent axillary nodes.

4 Discussion

Breast carcinoma is a curable disease if detected early. It metastasizes through lymphatic system; therefore, lymph node examination plays a pivotal role in staging work-up and extent of surgery in the breast cancer patients. Traditionally axillary node dissection (ALND) being done to assess the status of lymph node, but it leads to edema, shoulder stiffness, pain [21]. Hence, sentinel lymph node biopsy (SLNB) is the minimally invasive and well accepted procedure of node evaluation for treatment of breast cancer patients [22]. Sentinel lymph node is the first group of nodes draining the tumor bed [21]. Blue dye or isotope colloid or both used for detecting sentinel node [8]–[10]. Hence, Sentinel node can be defines as hot, palpable and blue node in the axillary tissue which receives lymphatics drainage in the blue lymphatics vessels [16]. In SLNB surgeons remove the first node that receives the lymphatic drainage from the tumor. SLNB procedure is available in the study of Qaiser *et al.* [23]. Blue dye and radio-isotope method have 95 % identification rate of sentinel node and false negative rate vary from 5% to 10 % [17]–[19], [24]. But dye guided method is safe, cheaper and can avoid radiation exposure then isotope guided method.

Mechanism of metastasis to lymph node was described by Kawada and Taketo [25]. When metastatic node were more than 2 mm on histological section such metastasis were classifies as “macro-metastasis” [26]. “Micro-metastatic” nodes were classified when nodes were measured 0.2 mm to 2 mm in diameter. When the size of tumor cell less than 0.2 mm then they are called “isolated tumor cells” [22]. Micro-metastasis and isolated tumor cells do not increase the chance of locoregional recurrence and does not affect the disease-free survival. However, macro-metastasis in the lymph node increases the risk of local recurrence. Thus, it is important to detect the lymph node containing macro-metastasis. Kahlert *et al.* [20] demonstrate that the growth of tumor (either animal or human) depends on angiogenesis. The process of angiogenesis can be understood from the figure 4.

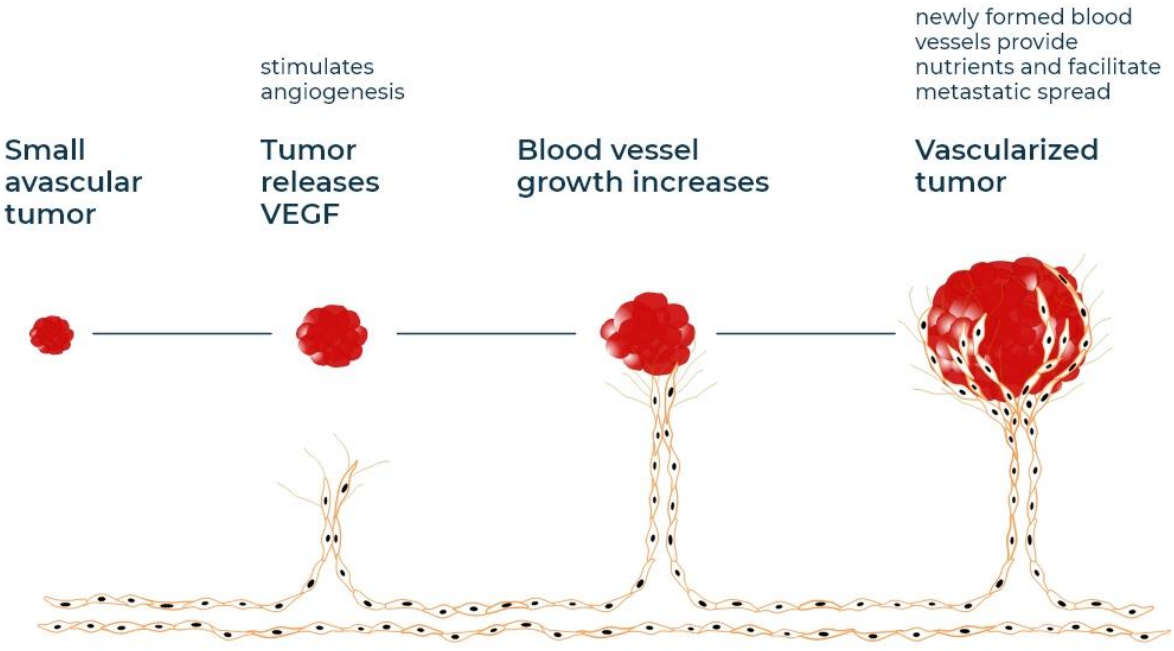


Figure 4: Process of angiogenesis

When tumor cells attain the diameter of 0.2-2 mm, they began to secrete "Vascular endothelial growth factors" - VEGF family (VEGF-A, B, C & D) [27]. Growth of cancer cells inside a sentinel lymph node is associated with neo-vascularisation. This neovesculatature of blood vessels have increase blood flow to the lymph node containing tumor cells of diameter > 1mm [28], [29]. This increased blood flow can be detected by Doppler ultrasound flowmetry [30], [31].

In the present study we used fluorescein as a fluorescence tracer. Intradermal injection of fluorescence tracer has some drawbacks. If lymphatics are block by the presence of cancer cells or following fibrosis due to previous surgery/radiotherapy, then tracer will not go to the lymphnode and tracer will bypass to the other uninvolved node and when node subject to the histopathological examination, report indicate false negative result while the cancer containing node is missed recurs later in the patient life. The false negative SLN mechanism can be understood by figure 5. So, we inject fluorescein intravenously instead of intradermal injection.

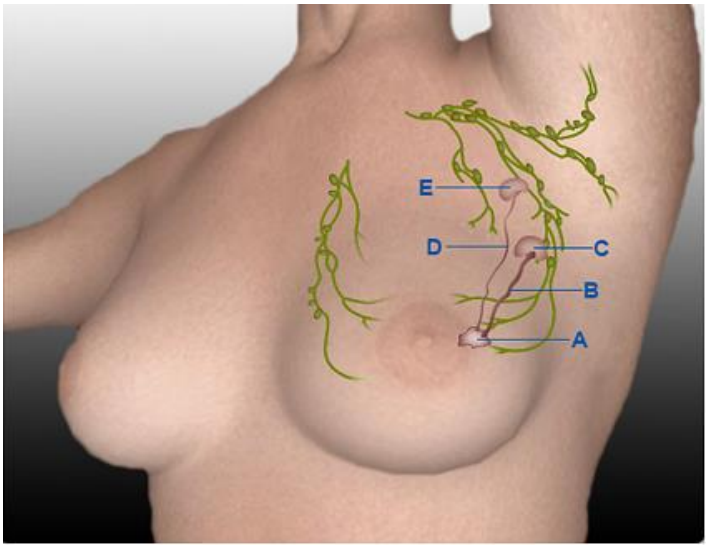


Figure 5: False negative lymph node due to lymphatic blockade by tumor embolus. (A) Breast tumor, (B) Lymphatic blocked with tumor embolus, (C) Lymph node with tumor metastasis, (D) Patent lymphatic carrying dye to non-sentinel lymph node, (E) Non-sentinel lymph node [16] .

Fluorescein sodium ($C_{20}H_{10}Na_2O_5$) is an organic dye with high quantum yield (o.93) [32]. When injected intravenously it is evenly distributed in the body. Circulating fluorescein bound to the albumin and this circulating fluorescein wash out rapidly from the body only extravasated fluorescein will stay in the extracelluar matrix for longer period. Fluorescein is an eye-catching fluorescent dye since, it has broad clinical experience therefore its side effects are well described [33]–[37]. secondly, small tumor can also be detected from this dye because it has high quantum yield. It is diagnostically best dye due to short time interval between the administration and extravasation. Fluorescein widely used to detect ocular diseases [38].

Since metastatic lymph node have increased blood flow which allow higher concentration of intravenously injected fluorescein will reached to the center of node. The anatomy of lymph node can be understood by the figure 6. In center of node intravenous blood reaches and at periphery (at capsule) only lymphatics blood. Therefore, node becomes more

fluorescent from the centre than the periphery when fluorescein injected intravenously (seen in figure 2).

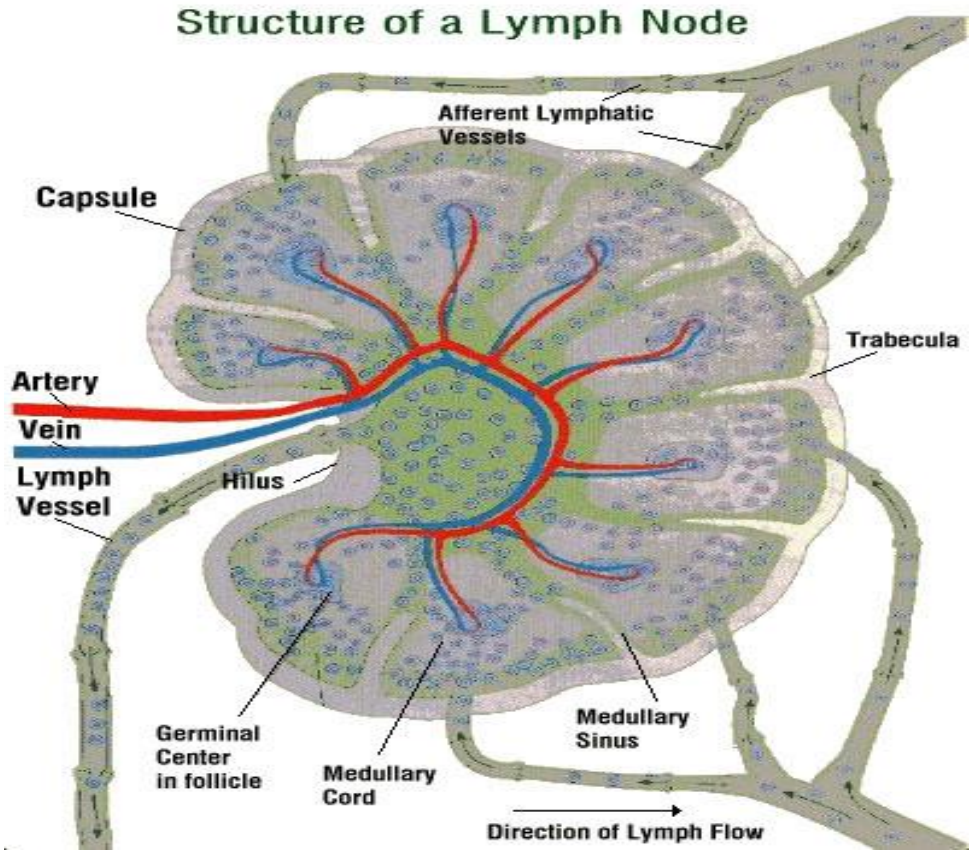


Figure 6: Anatomy of lymph Node

The tumor vasculature differs from normal vessels in being devoid of smooth muscles and precapillary sphincters and having large diameter vascular network with many arteriovenous communications. The blood flows through this vascular network at a high velocity with low impedance [23]. With the help of Fluorescein dye (Fluorescein angiography) tumor associated neo-vascularisation can be detected by naked eye immediately after intravenous injection of dye, as the increased blood flow through a leaky vascular network in the tumor and metastatic node would enable high concentration of fluorescein.

In the present study we found 185 fluorescent nodes and 82 non fluorescent nodes. Out of 185 fluorescent nodes 103 nodes were histopathological positive (or true positive) and remaining were negative on histopathological examination (HPE) (or False positive). From 82 non-fluorescent node only 6 nodes were containing tumor cells (or False negative) and remaining nodes are free of tumor (or True negative) The false positive results on HPE this may be due to since some patients received pre-chemotherapy. Since after chemotherapy, necrosis fibrosis changes appear which shows increased uptake of Fluorescein. Hence, these nodes were fluorescent but histopathological negative. Sensitivity and specificity of the method is 94.4% and 48%. Positive predictive value is 55.6%. Intravenous fluorescein technique is safe, non-toxic and is in good agreement with the conventional technique.

5 Conclusion

Intravenous fluorescein sodium has a high sensitivity of 94.4% & specificity of 48% with positive predictive value of 55.6% which is comparative to conventional sentinel LN procedure in detecting early metastasis. Besides time saving and non-toxic, this technique predicts the metastatic status of axillary lymph node during surgery, aiding appropriate surgical intervention.

6 Declarations

6.1 Acknowledgements

One of the authors (Darakhshan Qaiser) acknowledges University Grant Commission of India (UGC) for granting post-doctoral research fellowship.

6.2 Ethical Approval

The project was approved from institute ethical committee, All India Institute of Medical Sciences, New Delhi.

6.3 Informed Consent

An informed written consent was obtained from the participants.

6.4 Competing Interests

The authors declared that no conflict of interest exist in the publication of this work.

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