VOLUME-9, ISSUE-2, FEBRUARY-2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Forensic Science

A BRIEF REVIEW ON NON-INVASIVE RADIO IMAGING ADVANCES IN FORENSIC ENTOMOLOGY

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ABSTRACT Radio-diagnosis is an established field in clinical practice including postmortem Computed Tomography (CT) scans for better evaluation on the cause of death. Applications of micro CT scan on age-specific diagnostic marker in immature insects as well as volumetric estimation of maggot biomass from deep tissue for precise calculation of minimum Postmortem interval (PMI) is an upcoming challenging field. Proper interpretation of images with data refinements are presently a new forensic research field with advantages on saving of time and evidences properly.

KEYWORDS : Forensic Entomology, Micro Computed Tomography, Pupa, Maggot biomass, X-ray attenuation coefficient

1.INTRODUCTION:

Radiological diagnosis have an immense role in forensics ranging from anthropological age estimation, pattern study of injuries, gunshot wounds, presence of foreign bodies, comparison between ante and post-mortem images of skeletal remains, interpreting the mode of death and more. Cases of traumatic injuries, fractures, sources of intracranial hemorrhage, penetrating wounds and vascular lesions can be best interpreted with radiological corroborations. Cases of strangulations with fractures of the thyroid Cornu or hyoid bone can be easily discriminated with dislocation fractures related to vehicular accidents and therefore may assist investigators [1,2].

Moreover, cases with religious stigma or when the parties are not willing for conventional autopsy, virtual autopsies can be easily opted with quick decisions. With the advancements of Multi Detector Computed Tomography (MDCT), multiplanar studies can be interpreted non-invasively. Recently, Magnetic Resonance Imaging (MRI) technology has been introduced in various forensic investigations with precision result [1,2]

Imaging techniques have provided a valuable tool for forensic investigations of entomological evidences. This manuscript has been prepared based on systematic review of relevant literatures published since 2012 to 2019 [3-9] from different electronic databases viz. Pubmed, Google Scholar, etc. and extended with comprehensive knowledge to enumerate the applications of non-invasive radiotechniques in the field of forensic entomology. The aim of this review is to assemble relevant informations, analyze them and search for loopholes from previous works in this juvenile field so that they can be applied for future case studies.

2.Forensic age estimation of immature insects:

Age estimation of entomological exhibits are required at times beyond 72hrs of cadaveric age to know the exact time of death for forensic relevance. In such cases non-invasive 3D imaging techniques are useful over traditional histological stainings. Though the field is young, still authors have attempted to investigate the internal morphological changes of blowfly pupae (intra puparial development:IPD) along the time frame to establish a non-destructive approach towards proper estimation of minimum post mortem interval.

Richards et al. (2012) investigated the internal morphologies of the pupa of *Calliphora vicina* stained with aqueous 0.5 M iodine solution (standardized) for a preferred amount of time (1 day and 7 days) without any manual perforations. This differential staining methods were applied to standardize the optimal contrast for better image analysis. Samples stained for a longer period demonstrated standard resolution. Micro Computed Tomography scans (Micro CT scans) were processed using Nikon Metrology HMX ST 225 system where exposure target were set at 500ms with voltage approx. 80KV. The images were reconstructed using CT-Pro 2.1 software following noise reduction level 2 and manual centre of rotation. Virtual images were analysed using three principle planes sagittal, axial and coronal. Authors suggested that assessments of both internal and external features may assist in precise estimation of age [3].

Internal morphological changes during IPD of two necrophagous blowfly species *Calliphora vicina* and *Lucilia sericata* (Diptera: Calliphoridae) were studied analyzing 3D radiographic techniques. Development of two anatomical landmarks viz. the indirect flight muscles and alimentary canal were extensively studied on stained (standardized) pupa for demonstrating qualitative changes. Tomographic sections were critically analysed for both qualitative and quantitative measures at different percentages of time during the total IPD (100%) to precisely highlight the age-diagnostic marker. Authors highlighted that explosion of the gas bubble in most puparia at 10% stage of development due to hot water immersion can preclude those morphological analyses [4].

Internal morphological changes during IPD of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) has been extensively studied. *C. megacephala* were reared in laboratory conditions at 23.83±0.25°C with 12 hours of alternate lighting until pupal stage. Pierced Pupa collected at four different quarters of IPD (24, 48, 72 &96 hours) were stained for contrast enhancement by the protocol standardized after Richards et al. 2012. Tomographic scans were performed using Skyscan 1176 High Resolution In-Vivo Micro-CT with a 0.5mm Al filter and analysed with CT Analyser CTAN 3D software. Age-informative anatomical landmarks were mapped through coronal and sagittal planes [5].

3. Forensic estimates of Maggot biomass:

Dipteran larvae (maggots) play a significant role in taphonomic decomposition of bodily remains. The temperature of maggot biomass has been measured to be high above the ambient by a difference of 20° C and even rise to lethal value [6]. A study on single species aggregation (*Lucilia sericata*) with a minimum mass size of 1200

individuals showed significantly higher temperatures above the ambient (22° C) after 26 hrs [7].

Thus cyclic rotation of individual maggots within their biomass has been demonstrated to be their possible survival strategy. The center has been recorded to be the hottest with gradual decrease towards the periphery. Therefore, the critical evaluation for the amount of heat utilized by individual maggot to access their growth rate models are quite difficult in practical aspects. Again, neglecting such crucial factor and taking ambient temperature records into forensic account may lead to mistakes in estimating postmortem interval (PMI) through **Thermal Summation Model** [6,8].

Therefore, it is wise to consider the maggot biomass temperature for accurate estimation of time since death. For this, a probe may be inserted in the center, but that may lead to possible distortion of the actively feeding mass introducing problems. Slone and Gruner (2007) generated formulations to estimate the temperature from volume of surface biomasses. Authors highlighted that masses above 20cm^3 in volume have the capability of autonomous thermoregulations and this manipulation can impact on PMI estimation. However, masses smaller in size possibly develops by the ambient micro climate [6,9].

The problem continues, with the fact when maggot masses resides deep within the body or at internal spaces which are not easily approachable superficially.

4. Radio-imaging techniques in volume estimation:

Imaging techniques demonstrated for non-invasive volume estimation of maggot biomass within internal soft tissue, from early work of Johnson et al. [6] has got immense forensic value. To verify the application for volumetric estimation and incorporation of the same in case studies, a series of experiments were designed by the author as follows:

a. In vitro CT imaging of maggots and verifying the concept of radio-image techniques.
b. Impact of CT x- rays on survival and growth of maggots and understanding the fate of radiographic contrast (Isovue 300™, iopamidol compound) for volumetric estimations.
c. Estimating the size and volume of the mass by outlining the region of interest on the CT slice and minimizing the chance of error [6].

3.CT scan specifications for the best resolution of maggots: [6]

Name of the Author	Year of Publication	Type of CT scan	Software used	Workstation settings for the study
Johnson et al. [6]	2012	16 channel MDCT	Vitrea®2 imaging software	3D lung parenchyma option

MDCT: multidetector computed tomography [6]

4.Considerations from scanned images:

Notable features from experiments of Johnson et al. [6] are narrated as follows:

a) The view of individual maggots on longitudinal sections is curvilinear and on transverse section is ovoid in shape. The radiodensity value (HU) ranged between -50 and -250 of aggregated biomass is the radiologic feature for detection of a single species maggots (*Calliphora vicina*) within deep tissue despite of their movement which remain consistent over time.
b) CT x-rays with radiographic contrast (Isovue) did not have any significant impact on the growth and survival strategy of maggots. Though the radiation dose was not measured, the exposure rate considered during the examination may be compared with that of a routine CT scan of brain.
c) Axial, sagittal and coronal planes may be used for detection of maggots but axial view should only be accounted in limiting the boundary of biomass as per standard practice of radiodiagnosis. Satellite masses are to be traced thoroughly in all slices and linked with the main mass by drawing a thin line using an image software. In cases of true separate masses, they must be assessed independently. However, single maggots out of the mass are not considered under radiologic features. It should be noted that tissue as well as air pockets enclosed by maggot masses must be considered for image analysis where as they must be excluded from periphery to get a presice estimation. Radio images confidently identified as maggots within the estimated boundary of the biomass should only be considered under investigation whereas any foreign artifacts lying outside or even adjacent to the estimated boundary must be excluded.
d) Volume of the biomasses at four different volumetric sets (5,10,25 & 50) cm^3 were compared between known and experiments. By the authors, there were no significant differences between the studied sets except for masses of 10 cm^3 [6].

7. In search of the real cause for more precision :

Subsequent studies of Johnson et al. (2012) were addressed on maggot biomasses among mixed species from radiologic point of view. The study specifically looked upon the HU values for four maggot species of forensic importance viz. *Calliphora stygia*, *C. vicina* and *Lucilia sericata* , *L. cuprina* which revealed that Calliphora species (Diptera) had significantly less HU values compared to others. Maggot mass represents a heterogeneous environment predominantly comprising of maggots with trapped air pockets between tissue. This collectively represents the x-ray attenuation coefficient which is calibrated over HU scale. Thus the mixed model studied so far have different radiodensity values as demonstrated -43 to -526 for maggot masses as well as -805 to 729 for surrounding flesh. This may potentially incorporate confusions for HU values unless the images are properly analysed to identify maggots from their morphologies, as suggested by the author. However, the study highlighted the consistent negative range for HU values of maggot masses irrespective the surrounding tissue which is truly a noted fact [10].

Histological investigations were performed on sampled maggots of both the genera following standard procedures for hematoxyline and eosin counterstain. The results though did not show a significant differences in the percentages of fat, however, species from *Calliphora* (Diptera) has been demonstrated with higher fat content. This may be concluded as a remark on more trend towards negative x-ray attenuation coefficient in the study [10].

Density of maggot biomasses are regulated by certain principles-

i. Age and species of the maggot
ii. Fat content of the species
iii. Size of the mass
iv. Temperature and level of disturbance within the mass of interest that implies on how maggots orient themselves within the soft tissue that perhaps involve the amount of air pockets

On contrary, density of tissue in a decomposed body is also governed by certain factors-

i. The type of the tissue concerned
ii. Prevailing temperature that facilitates decomposition
iii. Postmortem interval

These cumulative factors are major attributes in considering and determining the regional x-ray attenuation coefficient, as suggested by the author [9].

8. CONCLUSION:

The paper highlights some of the notable extracted facts over

the application of non-invasive micro CT scan in forensic entomology from two broad aspects for better estimation of PMI viz. a) previous studies to evaluate the age-specific diagnostic markers from IPD and b) considerations from radio-image features on volume estimation of maggot biomasses – that may be incorporated in future casework in saving time and evidence.

Application of radio imaging techniques in age estimation of pupa has generated useful data in addressing the IPD for forensic relevance. Facts narrated from previous studies need to be verified at actual forensic casework to precisely estimate the PMI.

Virtual autopsy designed for visualization of maggot masses residing deep inside soft tissue can at times reproduce confounding results which is needed to be verified through extensive image analysis and expertise. Type of the tissue investigated may have an impact on outlining the maggot masses from calculated HU values (negative value), though it has been anticipated to be of less difficult from brain tissue which is positive (20-40 HU value). However, more researches are encouraged to rule out these facts.

Despite of previous studies this field is absolutely juvenile in its nature and therefore, there is a pressing need for data refinements and confirmations through extensive investigation relevant to medico-legal importance of entomological evidences from non-invasive, non-destructive approaches.

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