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Original Research

Cytopathological Evaluation Of Image Guided Fine Needle Aspiration Cytology Of Liver Lesion With Cell Block Correlation.

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Abstract:

Background and rationale: Image guided fine needle aspiration cytology of liver lesion is a widely used procedure, providing rapid and accurate diagnosis. However, exact diagnosis is not possible always with information obtained by fine needle aspiration (FNA) cytological material and the possibility of indeterminate diagnosis exists. Therefore, we attempted to obtain additional information via the preparation of Cell block (CB) from the residual material of aspirates to aid in increasing the diagnostic accuracy.

Objectives:

1. To study cyto-morphological features in various hepatic lesions
2. To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: Descriptive study

Methods and Material: We conducted a descriptive study at a tertiary care teaching hospital(Karnataka Institute of Medical Sciences, Hubli) for a period of one and half years which included a total of 75 cases with suspected liver mass. Using image guidance, fine needle aspiration was done and smears were prepared. The rest of the material present in the needle hub was submitted for cell block preparation.

Statistical analysis used: The quantitative variables were summarised using mean and qualitative variables as proportions. Sensitivity, specificity and positive predictive value were calculated for Image guided FNAC with Cell block as gold standard.

Results: Most common lesion was hepatocellular carcinoma. Sensitivity, specificity and diagnostic accuracy of smear preparation were 98%,90.2% and 96.6% respectively. With FNA and CB together, the sensitivity and specificity increased to 100%.

Conclusions: In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practices, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult to diagnose cases.

Key-words: Image guided FNAC, Liver lesions, Cell block

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

Introduction:
The liver is one of the common organs for various non-neoplastic and neoplastic lesions. These include primary liver tumours, secondary deposits, cysts, abscesses and granulomas.
Image guided fine needle aspiration cytology (FNAC) is widely used, rapid, cost effective and minimally invasive method for the diagnosis of the liver lesions[1,2]. In addition to conventional smears, an attempt to obtain additional information can be made via the preparation of cell block from residual material remaining after completion of cytology preparation. This material often contains tissue fragments, which can give valuable information that cannot be processed by cytology[1,3]. Cytopathological evaluation and cell block preparation from FNA together yield two differing, complementary view of the same cell population[2].
The present study is undertaken to evaluate the cyto-morphological features of various hepatic lesions in detail as well as to assess the utility of cell blockpreparation method in increasing the diagnostic accuracy.

Methodology:

Primary objective: To study cyto-morphological features in various hepatic lesions.

Secondary objective: To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: This is a descriptive study carried out in the cytology division of a tertiary care teaching hospital.

Study setting: Pathology Department of Karnataka Institute of Medical Sciences, Hubli, Karnataka.

Study duration: One and half years from October 2017 to March 2019 after obtaining the Ethical clearance from the Institutional Ethical Committee.

Study Subjects: Patients presenting with radiologically detected liver mass lesions were included in the study.

Exclusion Criteria: All abdominal mass cases other than liver lesion, patient with any bleeding disorder, non-co-operative patients and hydatid cyst diagnosed on ultrasound were excluded from this study.

Ethical considerations: Approval was obtained from institutional ethical committee prior to conducting the study(No.KIMS/PGS/SYN/447/2017-18).Informed written consent was obtained from each patient.

Method of data collection:
The patients were selected regardless of their age, sex, socio-economic status, and occupation. A detailed clinical history was taken and radiological findings were noted. Required tests like bleeding time, clotting time, prothrombin time and activated partial thromboplastin time were done. Under image guided techniques including Ultra-sonography (USG) and Computed tomography (CT), FNAC was done using lumbar puncture needle of 22 gauge. Minimum of 4 smears were prepared which included wet alcohol fixed smears for Haematoxylin & Eosin (H&E) and Papanicolaou's (PAP) stain, and air-dried smears for May-Grunwald Giemsa (MGG) stain. The rest of the material present in the needle hub and syringewere submitted in cell block solution (mixture of equal quantity of 100%

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

ethanol and 10% formalin) using needle rinse method. Centrifugation of the sample was done at 2000 rpm for 2-3 minutes. Cell button submitted for routine histopathological examination. Paraffin embedded;4-6-micron thick sections will be routinely stained with H&E stain. Whenever necessary, histochemical special stains and Immunohistochemistry (IHC)were used.

Statistical analysis:
The data obtained was analysed by SPSS 21.0 software trial version. Microsoft word and excel was used to assimilate the data and prepare the article. The quantitative variables were summarised using mean and qualitative variables as proportions.

Results:
A total of 75 cases with liver lesion, clinically or radiologically diagnosed, were investigated. Our study showed a wide range of age distribution ranging from 22-85 years. The mean age of presentation was 58.9 years. Incidence of non-neoplastic lesion were predominantly seen in 5th and 6th decades and neoplastic lesions were more common in 6th and 7th decades. Out of the total 75 cases, 69.3% were males and 30.6% were females.
Majority of the patient presented with complaints of pain abdomen (74.67%). Most common imaging technique used was USG (95%) and in the remaining cases samples were obtained using CT guidance.
Out of 75 cases, adequate material was obtained on FNAC in 70 cases (93.3%). Adequatematerial for cell block was obtained in 60 cases (80%).
Out of the 70 cases with adequate material, 56 were neoplastic and 14 were non neoplastic cases.
Non neoplastic lesions included diffuse parenchymal liver disease, pyogenic abscess, regenerative nodule and simple cyst. Among these 56 neoplastic lesions, 35 were Hepatocellular carcinoma (HCC) and 20 cases were metastatic deposits and the remaining 1 case was diagnosed as haemangioma [Table 1].

Table 1.Type of lesions.

Lesions	Diagnosis	Frequency
Non neoplastic	Diffuse parenchymal liver disease	07(10%)
	pyogenic abscesses	04(5.7%)
	Regenerative nodule	01(1.42%)
	Simple cyst	02(2.85%)
Neoplastic	Hepatocellular carcinoma	35(50%)
	Metastatic deposits	20(28.5%)
	Haemangioma	01(1.42%)

NON NEOPLASTIC LESIONS
Most common non neoplastic lesion was diffuse parenchymal liver disease. The predominant pattern of arrangement of hepatocyte in these caseswere clusters and sheets. All the cases showed intra cytoplasmic bile pigment and presence of bile duct epithelium. Out of the 7 cases,5 cases showed fatty change. Cell block section showed sheets and groups of benign hepatocytes having abundant cytoplasm, central round nucleus with fine granular chromatin and prominent nucleoli. Micro vesicular and macro vesicular fatty changes were seen in the 5 cases.Special stain Masson trichrome stain was used in a case of diffuse parenchymal lesion presented with history of cirrhosis and it demonstrated blue stained collagen amidst the hepatocytes. Smears of pyogenic abscesses showed predominantly sheets of neutrophils and nuclear debris in a necrotic background. Clusters of benign hepatocytes were present. Cell block sections showed sheets of inflammatory cells and groups of hepatocytes.

PRIMARY NEOPLASTIC LESIONS

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

Hepatocellular carcinoma was the most encountered lesion of the liver in the present study (50%).Detailed cyto-morphological analysis of each case was done and compared with cell block findings[Table 2].
Among 35 cases, aspiration was highly cellular in 26 cases and was moderate in remaining 9 cases. Out of the 35 cases, 20 cases showed classic trabecular pattern. It was the commonest pattern seen in well differentiated HCC. Other patterns commonly encountered were clusters and dispersed single cell arrangement. Clusters of hepatocytes with traversing blood vessels were seen in 30 cases (85.7%). Endothelial wrapping around the trabecula were seen in 15 cases (42.8%). Pleomorphic nuclei were observed in 88.5% of the cases. All the cases showed high nuclear cytoplasmic ratio, prominent macro nucleoli and atypical naked nuclei(100%). Intra nuclear cytoplasmic inclusion was present in 23 cases (65.7%). Presence of intracytoplasmic bile pigment could be appreciated in 8 cases (22.8%) and bile duct epithelium was absent in all the 35 cases.

Table 2. Cytomorphological analysis

Cytological feature	Number of cases
High cellularity	26 (74.3%)
Trabecular pattern	20 (57%)
Traversing blood vessels	30 (85.7%)
Endothelial wrapping	15 (42.8%)
High N/C ratio	35 (100%)
Pleomorphism	31 (88.5%)
Coarse chromatin	35 (100%)
Prominent nucleoli	35 (100%)
Naked nuclei	35 (100%)
Intra nuclear inclusion	23 (65.7%)
Intra cytoplasmic bile	08 (22.8%)
Absent bile duct epithelium	35 (100%)

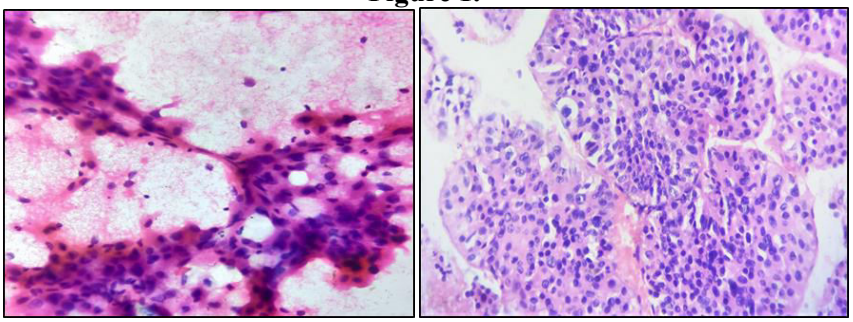
Hepatocellular carcinoma cases were further sub classified depending on these cytological and nuclear features. Out of these 35 cases, 8 were well differentiated HCC [Figure 1], 24 were moderately differentiated HCC and 3 cases were poorly differentiated HCC.

The well differentiated HCC showed high cellularity in majority of the cases with tumour cells arranged predominantly in trabecular pattern (75% of well differentiated HCC). These tumour cells were showing absent or mild pleomorphism with abundant granular cytoplasm, central round nuclei with coarse chromatin, prominent nucleoli and slightly increased nuclear cytoplasmic ratio. Endothelial wrapping was present in the 7 cases of well differentiated HCC (88%). Traversing blood vessels was present in 6 cases of well differentiated HCC (75%). The predominant pattern identified in cell block sections were trabecular pattern. Thickness of trabeculae was more than 3-4 cell thickness in all the cases.
The moderately differentiated HCC showed cellular smears with tumour cells arranged predominantly in clusters and in trabecular pattern. These tumour cells were moderately pleomorphic with high N/C ratio, round to oval nucleus with coarse granular chromatin, multiple macro nucleoli and moderate cytoplasm. Traversing blood vessels was present in 22 cases of moderately differentiated HCC (92%). Endothelial wrapping was present in the 8 cases of moderately differentiated HCC (33.3%). Cell block showed tumour cells arranged in small groups and trabecular pattern.
The cases of poorly differentiated HCC showed discohesive tumour cells arranged in dispersed singles. These tumour cells were having scant cytoplasm, marked nuclear pleomorphism and multiple macro nucleoli. Multi nucleated tumour giant cells were frequently observed. Cellblock sections showed dispersed tumour cells in singles and small groups.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

Out of 35 cases, 20 cases were confirmed with IHC using marker HepPar 1. These cases showed diffuse granular cytoplasmic positivity.

Figure 1.

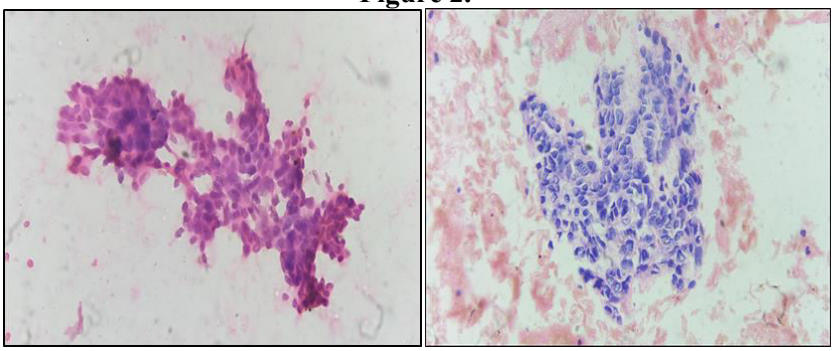


Well differentiated HCC (FNAC- H&E,40X,CB-H&E,20X)

METASTATICDEPOSITS

The remaining 20 cases out of the 56 neoplastic lesionswere reported as metastatic deposits to the liver. Of these, 18 cases were metastatic adenocarcinoma deposits [Figure 2], 1 case was metastaticadeno-squamous carcinoma deposit and the remaining 1 case was malignant melanoma deposit. Most common primary site of malignancy in this study was Colon (6 cases) followed by pancreas (4 cases), oesophagus (3 cases), breast (2 cases), ovary (1 case) and lung (1 case).

Figure 2.



Metastatic deposits from Papillary Carcinoma Ovary (FNAC-H&E,40x & CB-H&E,40x)

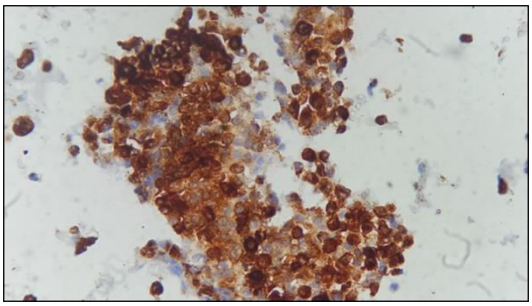
Most common pattern of arrangement of tumour cells observed in these metastatic deposits were clusters followed by acinar pattern and papillary pattern.

The present study predominantly comprised of metastatic adenocarcinoma deposits (90% of metastatic deposits). In adenocarcinoma deposits, tumour cells were cuboidal to columnar with hyperchromatic nuclei and moderate amount of vacuolated cytoplasm. Deposits from colon carcinoma showed intracellular and extra cellular mucin in the background. Cell block sections showed tumour cells arranged in clusters, acini and papillary pattern and having hyperchromatic nuclei and vacuolated cytoplasm. Special stain with PAS showed intracytoplasmic positivity.

Deposit from malignant melanoma showed large tumour cells with irregular nucleus, prominent nucleoli and moderate cytoplasm containing melanin pigments. Cell block sections showed sheets of tumour cells with intracellular and extra cellular melanin pigments. These cells showed strong cytoplasmic positivity with HMB-45 in immunohistochemistry [Fig 3]

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

FIGURE 3



IHC on cell block section showing intracytoplasmic positivity for HMB-45 in malignant melanoma

Out of the 20 cases, 3 cases were of unknown primary. Clinical details of these cases werecollected and an attempt was made to predict the primary site of tumour by assessing the cyto morphology in smears, histo-morphology and IHC in cell block [Table 3]

Table 3.Cytomorphology in smears, histomorphology and IHC in cell block a comparison

CLINICAL DIAGNOSIS	FNAC DIAGNOSIS	CB DIAGNOSIS	IHC
CT; pancreatic solid lesion	Metastatic adenocarcinoma deposits	Adeno squamous deposits	CK5 +, CK7+
Suspected case of primary Carcinoma stomach	Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	CK7+
Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	Cholangiocarcinoma	Inadequate

ANALYSIS OF EFFICIENCY OF SMEARS

Diagnostic sensitivity, specificity and positive predictive value of FNAC smears were calculated considering cell block as gold standard[Table 4].

Table 4. Diagnostic test evaluation parameters of FNAC

Diagnostic test parameter	Value
Sensitivity	98%
Specificity	90.2%
Positive predictive value	96.6%

Table 5.Comparison of cytological features of HCC in different studies

Cytological features	Shashikala V et al[12]	Cohen et al[19]	**Present study**
High cellularity	-	83%	**74.3%**
Trabecular pattern	50%	65%	**57%**
High N/C ratio	-	71%	**100%**

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

Naked nuclei	65%	73%	**100%**
Prominent nucleoli	100%	54%	**100%**
Intra nuclear inclusion	45%	-	**65.7%**
Intra cytoplasmic bile	15%	-	**22.8%**
Coarse chromatin	-	33%	**100%**
Pleomorphism	-	71%	**88.5%**
Absence of bile duct epithelium			**100%**

In 7 cases (10.3%) with difficult or doubtful diagnosis in smears, CB helped to arrive a definitive diagnosis. Difficulties faced in the present study was differentiating non neoplastic lesions from well differentiated HCC, differentiating poorly differentiated HCC from metastatic deposits and detection of the origin of metastatic deposits.Whichever cases had difficulties in differentiating the well differentiated HCC and non-neoplastic liver diseases using smears, we used cell block to confirm the diagnosis. Solid pattern and trabeculae of hepatocytes thicker than 4 cells rimmed by endothelial cells were considered as a feature of HCC.Differentiating poorly differentiated HCC from poorly differentiated metastatic tumour is challenging. IHC using Hep Par-1 antibody was performed in doubtful cases to confirm the diagnosis.
With FNA and CB together, we were able to provide a definitive cyto-pathological diagnosis in all the 60 cases, increasing the sensitivity to 100%.

Discussion:
Fine needle aspiration cytology of the liver was established by Sodenstorm in 1966 with the examination of 500 cases[4].Later in 1976, Haaga et al introduced a method of preciselocalization of lesion by Computed tomography (CT). This allowed accurate positioning of needle in small and deep lesions[5].Over the last 15 to 20 years, FNAC under image guidance has attained an increasing acceptance as the diagnostic procedure of choice for single or multiple focal hepatic lesion.
The use of cell block for processing cytology fluids has been first reported in 1895 by Bahrenberg[6,7]. In addition to the conventional smears, cell block technique has a significant role in diagnostic cytopathology and it is a valuable adjunct towards a more accurate cytological diagnosis complementary to smears and histology[6,7,8]. Cell block preparation are used routinely for body fluids, fine needle aspirations and other cytological samples.
Computed tomography(CT) and ultrasound guidance (USG) are the two main image guidance system using for liver FNAC. In this present study, ultrasonography was the main guidance technique we have used.
In the present study, the mean age at presentation was 58.9 years ranging from 22-85 years which is comparable with the study done by Haqsheefa et al[10] in which the mean age of presentation was 58.8 years. Maximum number of cases were seen between 51 and 70 years of age (6th and 7th decade) (70%) and this is comparable with the study done by Mathew and Nair[11] in 2017 and Shashikala V et al.[12] in 2016. Our study showed a male predominance with male to female ratio of 2.3:1 and these results are similar to the studies done by Haqsheefa et al[10] and Shashikala V et al[12].
Most important requirement for cyto-diagnosis is to obtain a representative sample[13]. In the present study, satisfactory aspirate for cyto-diagnosis on FNAC was obtained in 70 cases (93.3%). This is comparable to 91.67% adequacy obtained in a study done by Rajesh Chandan et al[14]and 96.96% adequacy obtained in the study done by Shashikala Vinayakamurthy et al[12].Adequate material for cell block was obtained in 60 cases (80%) and this is comparable to the study by Nathan et al.[15] who obtained adequate material on cell block in 73.3% cases. Major factor contributing to the optimal preparation of cell block is adequate rinses from fine needle aspiration syringe to extract the residual tissues.
Out of the 70 cases studied, majority of the lesions were neoplastic (80%) and the remaining 20% were non neoplastic lesions. This is similar to the distribution of cases found in the study by Balani et al.[13](2013), study by Rajesh Chandan et al.[14](2018) and study by Shashikala Vinayakamurthy et al.[12].

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

Most common non-neoplastic lesion identified in the current study was diffuse parenchymal liver diseases (10%). This is similar to the study conducted by Rajesh Chandan et al.[14]and the study conducted by Asghar F and Riaz S[16].In an another study conducted in Bangalore, Karnataka by Shashikala V et al[12], most common non-neoplastic lesion identified was pyogenic abscess.

The present study showed 50% of Hepatocellular carcinoma, 28.5% of metastatic carcinoma and 1.4% of haemangioma. Hence, the most common lesion of the liver in our study was hepatocellular carcinoma (HCC). This is comparable with different studies done by Shashikala Vinyakamurthy et al[12]., Mohammed AA et al.[1] and Sumana BS[3] in which the majority of cases were HCC. However, our study differs from the other studies done by Khanna et al.[17], Rajesh Chandan et al.[14] and Nosher et al.[18] since the majority of cases in their studies were metastatic deposits.

HCC is the most common primary malignancy of the liver and early diagnosis of HCC is important because of prognostic implication[10].Cohen et al.[19], proposed three primary useful criteria to discriminate between HCC and non- neoplastic liver lesion in FNAC. These features were increased nuclear cytoplasmic ratio (N/C), trabecular pattern and atypical naked nuclei.In the current study, we examined 10 cytological features that have been reported as useful in the literature for diagnosis of HCC. We analysed the utility of these features in all the 70 cases of liver FNAC. The current study identified the following features in all the cases of HCC; atypical naked nuclei in the background, presence of macro nucleoli, absence of bile duct epithelium, high N/C ratio and coarse clumped chromatin. These were the main features used to differentiate between HCC and non-neoplastic liver diseases in the present study. In addition to that, features like high cellularity and trabecular pattern are also considered. Useful features we found in the present study to differentiate between HCC and metastatic deposits are trabecular pattern, atypical naked nuclei, presence of macro nucleoli, intra nuclear inclusion and intra cytoplasmic bile pigment.

Based on above mentioned cytological features, all the cases of HCC were further sub-classified into 3 grades; Well differentiated HCC (8 cases), moderately differentiated HCC (24 cases) and poorly differentiated HCC (3 cases). This is comparable with the study conducted by Haq Sheefa[10] where majority of the HCC cases were moderately differentiated.However, one study conducted in Bangalore, Karnataka by Shashikala V et al[12] showed predominance of well differentiated HCC. Another study conducted in Bhopal by Balani et al[13] showed predominance of poorly differentiated HCC.

In the present study, metastatic deposits to the liver were accounting for 28.5% and major bulk of metastatic tumours in the present study comprised of metastatic adenocarcinoma deposits in 90% of metastatic deposits.This is comparable with the study done by Shashikala V et al.[12]and another study done in Bhopal, Madhya Pradesh by Balani et al[13], 89.6% of the metastatic deposits were adenocarcinoma deposits.The most common primary site of malignancy identified in the present study was colon and pancreas. This is comparable with the studies done by K.Ceyhan et al.[2] and Balani et al.[13]

The distinction between a primary carcinoma liver and metastatic deposits is very important since it has both therapeutic and prognostic significance.The cytological criteria to differentiate HCC from metastatic tumours stated by Bottles et al.[20] include polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal capillaries, presence of bile, intra-nuclear cytoplasmic inclusions and endothelial rimming.The salient features separating HCC from metastatic adenocarcinoma deposits described by Greene et al[22] were tumour cells in HCC are polygonal or polyhedral, have abundant eosinophilic and granular cytoplasm, macro nucleoli and trabecular arrangement.Whereas tumour cells in metastatic adenocarcinoma deposits are columnar or cuboidal, predominantly have acinar or glandular arrangement, show mucin secretions and inflammatory background.The salient features separating HCC from metastatic adenocarcinoma deposits identified in the current study were also the same.

Deposit from Metastatic melanoma may mimic HCC since tumour cells have several similar features. Also, melanin pigment can resemble various liver cell pigments. History of primary lesion elsewhere and features of melanoma like single cells with eccentric nucleus, single prominent nucleoli, bi-

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nucleation may help distinguish it from HCC. Immunohistochemistry can be done to confirm the diagnosis.

Various studies have reported sensitivity varying from 62-100% and specificity 63% to 100%[1,2,12,13]. In the present study FNAC was able to diagnose the liver lesion with an overall sensitivity of 98% and specificity of 90.2%. The overall accuracy of procedure in the present study was 96.6% which was comparable to the rate of accuracy reported in the studies done by Mohmmed AA et al[1] and Shashikala V et al[12].

The utility of cell block along with routine smears has been evaluated by various authors. In the present study, cell block provided additional information in 10.3% of the cases. In a study done by Liu et al[21], cell block section provided additional information in 12% of the cases andanother study by Nathan et al.[15], described 15.2% of improvement in diagnosis when both smears and cellblocks were studied together.In another study conducted in Bangalore, Karnataka by BS Sumana and Bharathi Muniyappa[3], cell block helped to improve the diagnosis in 15.55% of the cases.

With smear and cell block together, 100% correct diagnosis was achieved in all the 60 cases increasing the sensitivity and specificity to 100%. This is comparable with the study done by Shashikala V et al.[12] The result of this study revealed that diagnostic sensitivity can be increased by adding cellblock as adjunct method along with conventional smears.

Cell block sections displayed cytomorphology clearly recognizable with limited shrinkage. The cyto-morphological features were properly maintained with clear recognition of nuclear and cytoplasmic features. This is corresponding to study done by Kung et al.[23] regarding the staining results on CB where cell block sections showed excellent staining results with IHC.In addition to the role in increasing diagnostic sensitivity, cell block section can also be used for special stains and immunohistochemistry. In the current study, cell block sections showed excellent staining pattern with IHC markers.

Conclusions:

In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practice, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult cases.

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ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

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ORIGINAL RESEARCH

Effects of chronic oral sodium benzoate on glucose homeostasis using adult mice as experimental models

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Abstract

Introduction: Sodium benzoate (NaB) is a widely used food preservative and antimicrobial substance. Sodium benzoate is a major preservative in soft drinks. Large scale consumption of soft drinks is a risk factor for T_2DM. Studies have shown that sodium benzoate decreases leptin levels which can lead to diabetes mellitus. Since studies exploring the effects of chronic oral sodium benzoate on models of glucose homeostasis are lacking, we intend to evaluate and compare the effects of chronic administration of sodium benzoate on glucose homeostasis in experimental models of adult mice.

Materials and methods: The study aims to evaluate the effects of chronic oral sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis using adult mice as experimental models through a Prospective interventional animal experiment study and to evaluate the histological changes produced in the pancreas.

Results: Within-group analysis was performed from baseline to 6 months in the animals of test groups to detect any significant changes in the blood sugar values. Significant changes were noted after120 minutes (baseline versus after 6 months), the p-value was 0.045 which was statistically significant. There was no significant difference in other time frames. Among the 10 slides prepared from the pancreas of experimental mice of the test group, two slides showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues.

Conclusion: Sodium benzoate taken orally for 6 months at a dose of 62 mg/kg/day produced a significant change in glucose homeostasis. Insulin resistance was observed at 120 minutes as per IPGTT test. It also produced histological changes like reduction in islet cell number which may be due to oxidative stress. Long term, studies may be necessary to confirm these unfavorable effects and depending on the results of these studies there might be a necessity for the regulation of the use of sodium benzoate.

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Introduction

Sodium benzoate (NaB) is a widely used food preservative and antimicrobial substance in salad dressings, pickles, vinegar, carbonated drinks, jams, fruit juices, sauces and also present in medications and shampoo. Chemically it is the sodium salt of benzoic acid. The chemical formula of sodium benzoate is $C_7H_5NaO_2$[1].Sodium benzoate and Potassium benzoate are commonly used food preservatives that are listed among the "generally regarded as safe" (GRAS) compounds by the United States Food and Drug Administration and can be present in foods at a concentration up to 0.1%[2,3]. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) expert committee on food additives recommends an acceptable daily intake of sodium benzoate as 5 mg/kg body weight[4].

After ingestion of sodium benzoate rapid absorption occurs both in humans and animals. Studies showed that maximum plasma concentrations are reached within one to two hours[5].In the liver, the benzoate reaching through the diet is conjugated with glycine and will produce Hippurate. So, the deficiency of glycine is a limiting factor for the metabolism of sodium benzoate. This process mainly occurs in the mitochondrial matrix of the liver and kidneys. So, the intake of preservatives increases both benzoate and Hippurate levels in the body[6].

Animal studies have demonstrated that sodium benzoate given as oral gavage at a dose of 200mg/kg/day for 4 weeks showed anxiety and motor impairment in rats. Short term (4 weeks) consumption of sodium benzoate can impair memory performance and increase oxidative stress in mice[7]. Sodium benzoate is a major preservative in soft drinks. Large scale consumption of soft drinks is a risk factor for T_2DM. Studies have shown that sodium benzoate decreases leptin levels which can lead to diabetes mellitus. In a study done on acute exposure to GRAS levels of sodium benzoate, the results show that sodium benzoate does not affect insulin and glucose homeostasis, but further studies will be necessary to explore the metabolic impact of chronic benzoate exposure[8]. A study conducted in patients with renal insufficiency showed that the metabolite of sodium benzoate, Hippurate can accumulate and impair basal and insulin-stimulated glucose uptake into cells in culture[9]. This study concluded that this could explain the altered glucose homeostasis observed in these patients[10]. Another study showed a contradictory result where high dose intravenous sodium benzoate produced Hyperglycemia[11].Since studies exploring the effects of chronic oral sodium benzoate on models of glucose homeostasis are lacking, we intend to evaluate and compare the effects of chronic administration of sodium benzoate on glucose homeostasis in experimental models of adult mice.

Glucose intolerance can be defined as dysglycemia that comprises both prediabetes and diabetes. It includes the condition of impaired fasting glucose and impaired glucose tolerance and diabetes mellitus.

Methodology

Our study aims to evaluate the effects of chronic oral sodium benzoate on glucose homeostasis using adult mice as experimental models.

Primary objective: To evaluate the effects of chronic oral administration of sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis using adult mice as experimental models

Secondary objectives: (1). To evaluate the histological changes produced by chronic oral administration of sodium benzoate at the dose of62 mg/kg/day in the pancreas of adult mice.

(2). To evaluate the effects of sodium benzoate on insulin resistance by Intraperitoneal glucose tolerance test (IPGTT) &Intraperitoneal insulin tolerance test (IPITT).

Sample size: Calculated using G* POWER ® version 3.1.9.2, by assuming an α of 0.05, βof 0.05 and power of 95%. Assuming a 10% attrition rate, the corrected sample size was calculated using the formula [sample size /1-(attrition/100)10]. Ten animals in a group are required to detect a statistically significant difference between the groups and within the group.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

Ten animals are needed in the negative control group to detect changes between the groups to avoid bias due to individual variation. So totally of 20 animals were required for this study.

Animals: In this experimental study, 20 three-month-old male Swiss albino mice weighing 20 to 40 g were used, which were obtained from Sree Chitra Thirunal Institute of Medical Sciences & Technology, Biomedical Technology Wing, Satelmond Palace, Poojappura, Trivandrum, Kerala - 695 012. Reg No: 98/GO/R-SL/BiS/99/CPCSEA. The study got approval from the institutional ethics committee, Sree Gokulam Medical College, Venjaramoodu Trivandrum. (SGMC-IAEC No.005/08Am/M4/2019). The animals were kept and experiments were performed at the animal house of Sree Gokulam Medical College, Venjaramoodu, Trivandrum. The stabilization period (Quarantine) was 10 days. The mice were caged in polycarbonate boxes in stipulated environmental conditions. The animals were given pellet chow and filtered water ad libitum. The mice were placed in two groups of 10 each. (Control and test group). Each animal in the cage has got a unique id for its identification.

Study Design- Prospective interventional animal experiment study.

Study Setting-Animal house of Sree Gokulam Medical College & Research Foundation, Venjaramoodu, Trivandrum-695607

Duration Of Study-Study commenced on 20/05/2019 after obtaining approval from the institutional animal ethics committee. The total study duration was12 months.

Experimental Design

In this prospective interventional study, 20 animals were divided into two groups. The Control group (n=10) received a chow diet and filtered water ad libitum daily for six months. The Experimental Group (n=10), received a daily dose of 62 mg/kg for 6 months via drinking water along with a standard laboratory diet.

Table1: Experimental Design

Group	Daily administration	Route	Duration
Control(n=10)	Filtered water + Chow diet	Oral	6 Months
Test, n=10	Filtered water + Chow diet+ Sodium Benzoate 62 mg/kg	Oral	

Dose calculation of sodium benzoate

Acceptable Daily Intake (ADI) in humans =5mg/kg body weight per day, Conversion factor =12.3, Mice Equivalent dose =12.3 x 5=61.2 mg/kg. Sodium benzoate was given at a dose of 62 mg /kg/day dissolved in drinking water. The daily average consumption of drinking water for each mouse was assessed during the initial quarantine period as 7ml. The required amount of sodium benzoate (62 mg/kg) was dissolved in the estimated quantity of drinking water and given daily orally over 6 months.

The average body weight of mice in the test cage is calculated as 39.2 g in the test 1 cage and35.2 g in the test 2 cage. The required amount of Sodium benzoate was calculated for each group and dissolved in the drinking water. The dose calculations are given below.

The required amount of sodium benzoate to be given =62mg/kg/day

1 kg →62mg

1000g→62mg

1g→62/1000mg

39g→62/1000 X 39= 2.418 mg (For test 1 cage)

2.418 mg of sodium benzoate dissolved in 7 ml of drinking water

In 7 ml →2.418 mg

1ml=2.418/7

1/1000 L=2.418/7 mg

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

1L=2.418/7 X 1000=345 mg dissolved in 1 L of water
35g→62/1000 X 35=2.17 mg (For test 2 cage)
In 7 ml →2.17 mg
1ml=2.17/7
1/1000 L=2.17/7 mg
1L=2.17/7 X 1000=310 mg dissolved in 1 L of water

Data Collection Procedure

After the initial quarantine and grouping, the health parameters were recorded. Monthly weight recorded in the animal register. The health of the animals was monitored with the help of a veterinary doctor. Blood sugar levels of all animals were monitored every month using a glucometer. Tests for insulin resistance such as Intraperitoneal Glucose Tolerance Test and Intraperitoneal insulin tolerance test was done at the baseline and the end of the study. Animals were sacrificed by CO2 inhalation according to euthanasia protocol approved by the IAEC.

Glucose Analysis: Blood glucose levels were measured at baseline and every month for 6 months by a glucometer. Blood glucose was measured using a one-touch Verio flex blood glucose monitor.

Tests for insulin resistance

1. Intraperitoneal Glucose tolerance test (IPGTT)

After 12 hours fasting, D-(+)- Glucose solution (analytical grade from a local vendor), 2g/kg BW was injected into the mice intraperitoneally and blood glucose levels were measured at 0, 15, 30, 60, and 120 minutes of glucose load using a glucometer. This was done at baseline and the end of 6 months[12]. The procedure steps are given below

20 % Glucose solution preparation[13]

20 g glucose dissolved in 100 ml of distilled water, Volume of IP Glucose injection (microliter) =10 x Body weight (gm) =10 x 35=350 microliter=0.35 ml. This was given intraperitoneally to mice and blood glucose levels were measured using a glucometer at 0, 15, 30, 60 and 120 min after glucose injection.

2. Intraperitoneal insulin tolerance test (IPITT)

Mice would have fasted for 6 hours. Huminsulin regular 40 IU/ml (Lilly company) purchased from the hospital pharmacy, stored at 2-8° C) was given intraperitoneally at a dose of 0.75 U/kg body weight. Blood glucose levels were measured using a glucometer at 0, 15, 30 and 60 min after insulin injection. This was done at baseline and 6 months[12].There was no evidence of an allergic reaction to human insulin in mice. The procedure steps are given below

Weight of each mouse in test cage is estimated
Dose to be given=0.75 IU/kg or 0.00075 IU/gm
The body weight of each mouse is substituted to get the required volume of insulin. If the body weight of a mouse is 35 gm, then the required volume of insulin is calculated as
35 g→0.00075 X 35 =0.026 u insulin
The insulin used for our experiment was Huminsulin Regular 40 IU/ml
1 ml contains 40 units,0.25 ml contains 10 units
0.25 ml insulin +9.7 ml NS contain 10 units
10 ml=10 u ,1ml =1 u
1 ml + 9ml NS = 1 U
10 ml=1u
0.26 ml of 10 ml was taken to get 0.026 U of insulin. This was given intraperitoneally to mice and blood glucose levels were measured using a glucometer at 0, 15, 30 and 60 min after insulin injection.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

Fig 1. Illustrative representation of the GTT experiment(A1) Materials and equipment required for GTT (D- glucose, 1-mL syringes, 25–27-G needles, microsurgery scissors, glucose test strips, glucometer, lab balance).(A2) Material and equipment required for ITT (Insulin, sterile saline, 1-mL syringes, 25–27-G needles, microsurgery scissors, glucose test strips, glucometer, lab balance). (B) Animals are weighed before the beginning of the experimental procedure for the estimation of proper amount of Glucose/Insulin. (C) i.p. injection. (D) Evaluation of blood glucose at different time intervals.

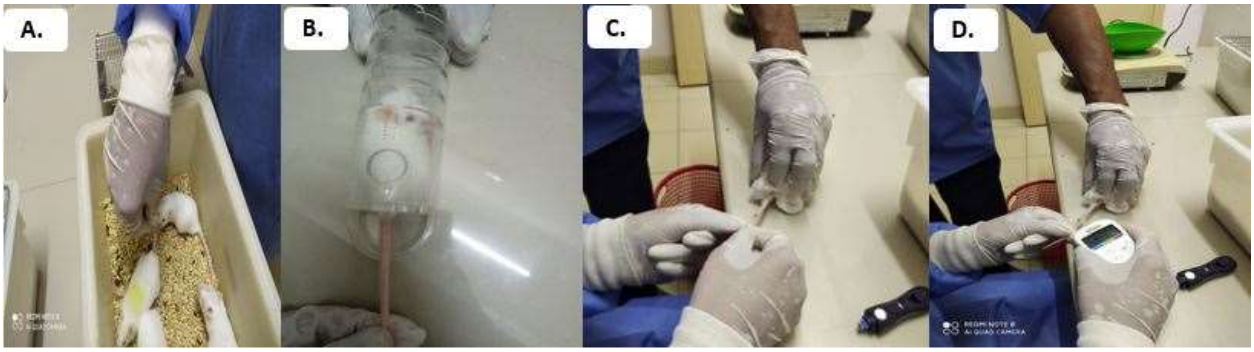

Figure 2.Illustration of blood collection procedure. (A) The blood of the mice to be estimated was identified and taken out from cage. (B) Identifying the lateral tail vein. (C)Pricking the vein with lancet.(D)Annotating glucose with glucometer strip.

Euthanasia

After the completion of experiments, animals were sacrificed using the CO_2 within the euthanasia chamber and tissues (Pancreas) was collected for histopathology studies. The procedure was done as per the recommendations by CPCSEA.

Histological Examination

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

Figure 3. Identifying pancreatic tissue for Histopathology

Pancreatic tissue samples were fixed with 4% neutral buffered formalin, dehydrated, and embedded in paraffin. Embedded tissues were 5-micrometre thickness and stained with hematoxylin and eosin (H&E). Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. At least 25 different areas of each pancreas slide were observed, and all islets were counted.

Statistical Analysis

The glucometer results were entered in the Microsoft Excel sheet and analyzed with SPSS version1.0.0. 1406.The data were compared within groups and with the negative control using an independent sample **t-test**. For all statistical interpretations, $p<0.05$ was considered as the threshold for statistical significance. Normality was tested using Shapiro- Wilk test.

Results

Our prospective animal experimental study evaluated the effects of chronic oral administration of sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis, on insulin resistance by Intraperitoneal glucose tolerance test (IPGTT)&Intraperitoneal insulin tolerance test (IPITT)using adult mice as experimental models. The histological changes produced in the pancreas of adult mice were also evaluated.

a) Evaluation of the long-term effect of Sodium benzoate on glucose homeostasis

Between-group analysis at the baseline-The comparison between blood sugar values of control and test group animals at baseline was done and was found to be non-significant. As shown in table no 2

Table No 2: Comparison of blood sugar values between Control group and test groupat baseline

At baseline	N	Mean	SD	t-value	p-value
Control	10	124.7	12.91	0.731	0.220
Test	10	119.5	18.95		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis of control groups-The comparison between blood sugar values of control group animals at baseline and after 6 months was done and was found to be non-significant. This has been done to rule out any changes in blood sugar values in the control group animals which can occur other than sodium benzoate administration such as age, stress etc. and is depicted in table no 3.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

Table No3: Comparison of blood sugar values between Control group at baseline and after 6 months

Control Group	N	Mean	SD	t-value	p-value
Baseline	10	124.70	12.91	-0.093	0.927
After 6 months	10	125.30	15.86		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis of test groups-The comparison of blood sugar values of test group animals at baseline and after 6 months was found to be non-significant and is depicted in table no: 4.

Table No4: Comparison of blood sugar values between Test group at baseline and after 6 months

Test Group	N	Mean	SD	t-value	p-value
Baseline	10	119.50	18.95	0.177	0.870
After 6 months	10	118	19.03		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group analysis after 6 months-The comparisons of blood sugar values between the control group and test group after 6 months was also done and found to be non-significant. Results are given in table no 5

Table No 5: Comparison of blood sugar values of Control group and test group after 6 months

After 6 months Group	N	Mean	SD	t-value	p-value
Control	10	125.30	15.86	0.932	0.420
Test	10	118	19.03		

Independent sample t-test, p>0.05 considered as statistically not significant

b) Tests for insulin resistance

IPGTT

Between-group analysis of IPGTT at baseline-Comparison of blood sugar values of IPGTT between control and test group animals at baseline were done and showed no statistical difference. Results are depicted in table no 6

Table No6: Comparison of blood sugar values of IPGTT at baseline

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	89.80	12.01	-0.602	0.254
Control	10	93.40	9.37		
After 15 minutes					
Test	10	203.30	17.12	-0.749	0.679
Control	10	194.6	13.67		
After 30 minutes					
Test	10	128.70	19.28	-0.106	0.681
Control	10	137.70	17.09		
After 60 minutes					
Test	10	106.70	16.28	-0.506	0.450
Control	10	109.90	12.81		

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

After 120 minutes					
Test	10	87.10	11.02	-0.665	0.454
Control	10	89.70	12.01		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group analysis of IPGTT after 6 months- Comparison of blood sugar values of IPGTT between control and test groups after 6 months given in table no 9. The mean score of the test group was 89.80±12.01 and the control group was 87.20±14.10 at 0 minutes. The difference between groups was statistically not significant (p>0.05). The comparison of blood sugar after 15 minutes,30 minutes ,60 minutes and 120 minutes also showed no significant change. The results are shown in table number 7.

Table No7: Between-group analysis of IPGTT after 6 months

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	89.80	12.01	0.44	0.662
Control	10	87.20	14.10		
After 15 minutes					
Test	10	203.30	17.12	0.728	0.476
Control	10	197.8	16.67		
After 30 minutes					
Test	10	128.70	19.28	0.112	0.912
Control	10	127.70	20.71		
After 60 minutes					
Test	10	106.70	16.28	0.719	0.481
Control	10	100.60	21.31		
After 120 minutes					
Test	10	87.10	11.02	0.418	0.681
Control	10	84.80	13.48		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis was also performed from baseline to 6 months in the animals of test groups to detect any significant changes in the blood sugar values. Significant changes were noted after120 minutes (baseline versus after 6 months), the p-value was 0.045 which was statistically significant. Given in table no 8.

Table No8: Within Group analysis of IPGTT

Group	N	Mean	SD	t-value	p-value
Test 0Min- Baseline	10	89.80	12.01	-0.603	0.256
Test 0min -After 6months	10	92.70	9.33		
Test 15Min- Baseline	10	203.30	17.12	-0.849	0.779
Test 15min -After 6months	10	209.30	14.36		
Test 30Min- Baseline	10	128.70	19.28	-1.12	0.782
Test 30min -After 6months	10	138.10	18.09		
Test 60Min- Baseline	10	106.70	16.28	-0.607	0.550
Test 60min -After 6months	10	110.80	13.81		
Test 120Min- Baseline	10	87.10	11.02	-0.881	0.045 *
Test 120min - After 6months	10	90.70	6.75		

*** Independent sample t test, p<0.05 considered as statistically significant**

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

IPITT

Between-group comparison-the comparison of blood sugar values of IPITT between the control group and test group was done at baseline using an independent sample T-test. But no significant change was noted after 0,15,30and 60minutes of the IPITT test. Depicted in table no 9.

Table No9: Between-group analysis of IPITT result at baseline

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	129	10.11	-2.09	0.051
Control	10	137.30	7.40		
After 15 minutes					
Test	10	75.90	8.16	-0.835	0.415
Control	10	83.80	28.76		
After 30 minutes					
Test	10	64.10	9.65	-0.791	0.44
Control	10	67.20	7.77		
After 60 minutes					
Test	10	62.50	7.41	-0.254	0.803
Control	10	63.60	11.53		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group comparison of blood sugar values of IPITT between the control group and test group after 6 months of the experiment using independent sample T-test. But no significant change was noted after 0,15,30and 60minutesof the IPITT test. As shown in table no 10.

Table No 10: Between-group analysis of IPITT result after 6 months

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	132.30	11.92	-0.667	0.454
Control	10	130.20	10.91		
After 15 minutes					
Test	10	73.50	8.64	-0.991	0.315
Control	10	76.90	9.38		
After 30 minutes					
Test	10	64.50	9.65	-0.243	0.801
Control	10	67.70	7.77		
After 60 minutes					
Test	10	62.50	7.41	0.538	0.758
Control	10	66.70	6.75		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis -Comparison of blood sugar values between test groups at baseline and 6 months was done to detect any significant changes in the control group and test group. But no significant change was noted. As given in Table 11.

Table No 11: Within-group analysis of IPITT

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

Group	N	Mean	SD	t-value	p-value
Test 0 min – Baseline	10	129.00	10.11	-0.667	0.454
Test 0 min -After 6 months	10	132.30	11.926		
Test 15 Min- Baseline	10	75.90	8.185	0.638	0.858
Test 15 Min-After 6 Months	10	73.50	8.644		
Test 30 Min- Baseline	10	64.10	9.655	-.083	0.636
Test 30 min- After 6 months	10	64.50	11.693		
Test 60 Min- Baseline	10	62.50	7.412	.000	0.160
Test 60 Min- After 6 Months	10	62.50	11.825		

Independent sample t-test, p>0.05 considered as statistically not significan

Histopathology result

Histopathology was done to detect the effect of sodium benzoate on pancreatic tissue. Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. Among the 10 slides prepared from the pancreas of experimental mice of the test group, two slides showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues. The control and test slides are depicted in figure No: 8.

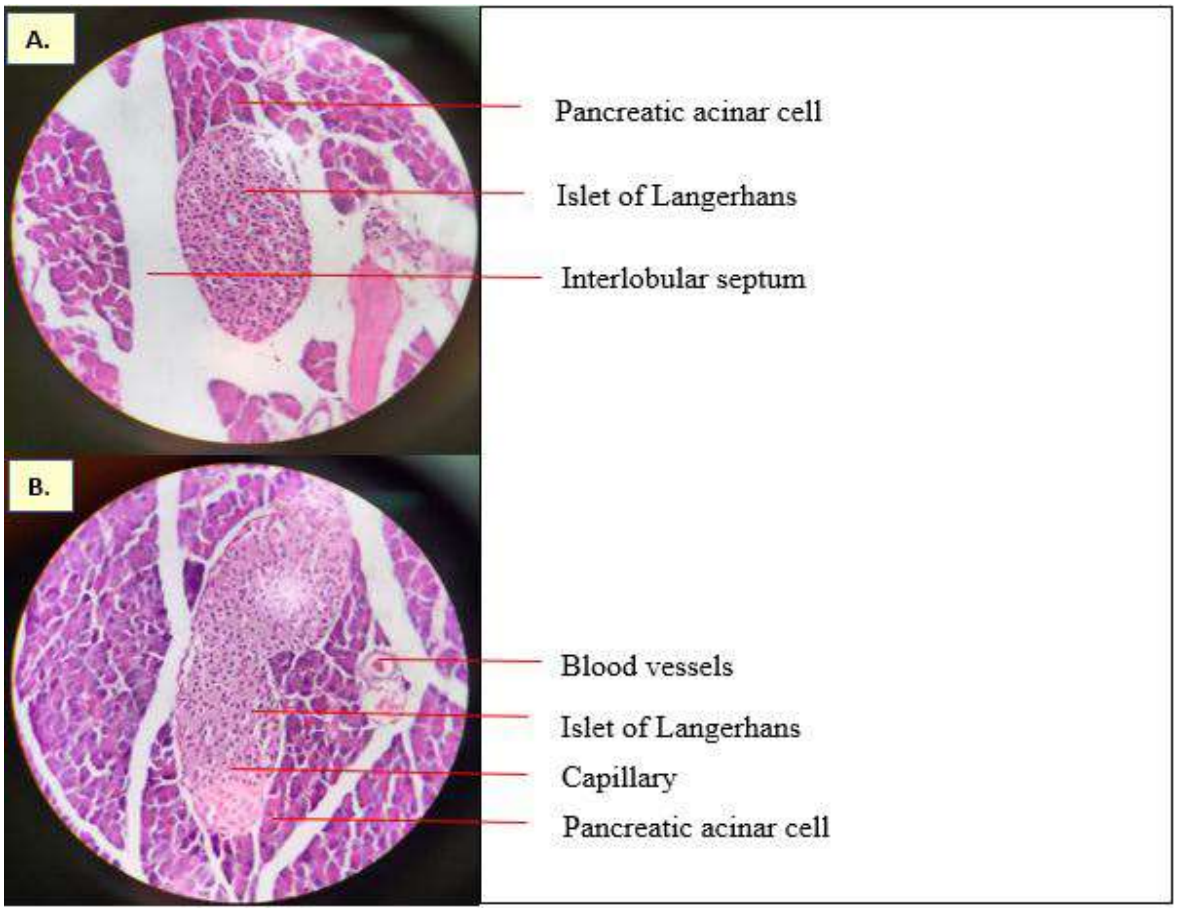

Figure 4. High power view of histopathology of control mice(A) and test mice (B). Normal Features such as Islet of Langerhans, acinar cells, blood vessels are seen. Fibrosis, Amyloidosis etc. which are features of Diabetes are not seen.

Discussion

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

In the present study, we evaluated the effect of sodium benzoate administration on glucose homeostasis using adult Swiss albino mice as experimental models for 6 months. The dose used in our study was 62 mg/Kg /Day. In addition to this, we also evaluated the histological changes induced by sodium benzoate in the pancreatic tissue of mice. Insulin resistance was evaluated by two tests IPGTT and IPITT. The between-group analysis of test and control was done at baseline and after 6 months. Within-group (comparison of blood sugar values of test group at baseline and test group after 6 months) analysis was also done. Statistically significant changes were observed in the IPGTT test at 120 minutes. No significant changes were noted in IPGTT at other time intervals. There were no statistically significant changes observed in the results got from IPITT. Short term studies conducted on sheep models showed analogues of benzoic acid can cause increase in plasma glucose concentrations[11].This study concluded that the endocrine pancreas could recognize the benzoic acid chemical structure and which will induce insulin and glucagon secretion in sheep. A clinical study of 14 days duration showed that oral sodium Benzoate administration doesn't produce any significant changes in insulin and glucose homeostasis[8].

Insulin resistance was tested by IPGTT and IPITT. In IPGTT test, baseline measurement of blood sugar showed no statistically significant differences between control and test groups (between groups). It was repeated after 6 months. The comparison of blood sugar after 0, 15 minutes,30 minutes,60 minutes and 120 minutes were done for detecting any significant change between groups. No significant changes were noted. Within-group analysis was also performed comparing the sugar values of the test group at baseline and test group after 6 months from baseline to 6 months to detect any significant changes in the blood sugar values. Significant changes were noted at 120 minutes. There were no significant changes in other time intervals (baseline versus after 6 months). The comparison of the results from the IPITT test was done between-group and within-group using an appropriate statistical test. But no significant change was noted after 0,15,30 and 60minutes of the IPITT test. IPGTT is used to assess the body's ability to metabolize glucose[14]. There is a lack of studies that assess the effect of sodium benzoate on insulin resistance which is usually assessed by IPGTT and IPITT. In our study, we evaluated the effect of sodium benzoate on glucose homeostasis and found that the 6-month administration of sodium benzoate produced significant change in glucose homeostasis. Further long-term studies are needed to confirm this.

Histopathology was done to detect the effect of Sodium benzoate on pancreatic tissue. Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. Two slides among test groups showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues. In a clinical study carried out in human lymphocytes, oxidative stress caused by sodium benzoate has been pointed out for the pancreatic beta-cell damage[15]. Another study conducted in Zebrafish larva showed that sodium benzoate could induce oxidative stress and the study suggested caution in excessive use of this preservative in processed and packeted foods[16]. In our study, two slides among test groups showed degenerative changes.

Clinical studies of short-term duration showed that oxidative stress can be developed as a result of sodium benzoate. This oxidative stress is implicated in the development of pancreatic beta-cell damage and insulin resistance[15]. Antioxidants are the agents that protect our bodies against various harmful oxidants. Superoxide Dismutase enzyme, catalase, glutathione peroxidase, glutathione – s- transferase, glutathione reductase are examples of antioxidant enzymes that present in our body and these enzymes protect our body against oxidative stress. A previous study showed that sodium benzoate administration reduced Glutathione peroxidase, glutathione -S- transferase, Catalase and Superoxide Dismutase enzyme levels[17]. This study also showed that sodium benzoate produces a significant rise in the levels of inflammatory cytokine markers such as TNF-α, IFN-γ, IL-1β and IL-6. This all may contribute towards the

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

development of oxidative stress followed by gland damage which could account for glucose homeostasis abnormalities.

Preservatives are added to the food to prevent microbial growth and undesirable chemical changes. But in the present century because of Industrialization, the growing fast-food culture and the lack of stringent regulations, the use of preservatives has increased a lot. But these compounds are toxic and was found to be harmful to humans. Sodium benzoate is one of the most commonly used food preservatives and is found in food items such as vinegar, carbonated drinks, jams, fruit juice, and condiments[15].FDA allows usage of sodium benzoate at 300 mg/1 kg in dairy products such as yoghurt, ice creams and pudding. But an increased dose of sodium benzoate taken for a longer period can lead to health disorders.

Our study concluded that 6-month oral administration of sodium benzoate produced statistically significant changes on glucose homeostasis, on IPGTT done for insulin resistance at 120 minutes. It also produced islet cell atrophy in 2 slides among the test groups. So further long-term studies are required to prove the effect of sodium benzoate as it is one of the major food preservatives used in the present decade.

Conclusion

Sodium benzoate taken orally for 6 months at a dose of 62 mg/kg/day produced a significant change in glucose homeostasis. Insulin resistance was observed at 120 minutes as per IPGTT test. It also produced histological changes like reduction in islet cell number which may be due to oxidative stress. Long term, studies may be necessary to confirm these unfavorable effects and depending on the results of these studies there might be a necessity for the regulation of the use of sodium benzoate.

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Histopathological Features and Clinical Variants of Biopsy Confirmed Psoriasis Cases in a Tertiary Care Setting in Kerala

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ABSTRACT

BACKGROUND

Psoriasis is a chronic, immune mediated, relapsing, papulosquamous disease having a high prevalence. Since it affects other organ systems such as musculoskeletal system, gastrointestinal system and the eye, it can lead to considerable disability. Although only rarely life threatening, it has high morbidity due to its chronicity and absence of cure.

METHODS

This study was conducted over a period of 2 years in the Department of Pathology, Medical College, Thiruvananthapuram. A total of 217 skin biopsy specimens in which a clinical diagnosis / differential diagnosis of psoriasis was made, was studied during this period.

RESULTS

108 cases out of 217 which were histopathologically diagnosed as psoriasis were studied in detail. Male predominance was noted in the study population. The mean duration of disease in this study was 6.69 yrs. Fifty percentage of the patients had associated comorbidities with hypertension outnumbering others. Among male patients, 26 (32.5 %) had the habit of smoking. The most common presentation was as erythematous scaly plaques, with pruritus being the second most common presentation. Histopathology proved to be conclusive of psoriasis in all cases. Hyperkeratosis was seen in all cases which was the most consistent histopathological feature. Confluent parakeratosis which is one of the characteristic features of psoriasis was seen in 62 (57.4 %) cases with the rest being focal. Other epidermal features studied were papillomatosis, hypogranulosis, suprapapillary thinning, and basal mitotic figures. Spongiosis was seen in 83 (76.9 %), exocytosis of neutrophils in 66 (61.1 %) and Munro's micro abscess in 42 (38 %) cases. Dilated blood vessel was the most common dermal change observed, seen in 105 (97 %). Lymphocytes were the most frequent upper dermal inflammatory infiltrate observed. Oedema was seen in 5 (4.6 %) of cases.

CONCLUSIONS

Psoriasiform lesions pose diagnostic dilemma to the treating clinician. To provide a clear-cut diagnosis, histopathological evaluation is essential. It is also important to differentiate between the different variants of psoriasis in the context of treatment. It has an important role in the follow up of psoriatic patients.

KEY WORDS

Psoriasis, Papulosquamous, Erythematous Scaly Plaques, Confluent Parakeratosis, Regular Acanthosis, Hyperkeratosis

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BACKGROUND

Psoriasis is a chronic immune mediated lifelong disease that primarily affects the skin. Since it affects other organ systems such as musculoskeletal system, gastrointestinal system and the eye, it can lead to considerable disability. Although only rarely life threatening, it has high morbidity due to its chronicity and absence of cure. It affects the quality of life as patients are embarrassed by their appearance, have reduced levels of employment and income. Psoriasis is a relapsing papulosquamous dermatitis characterized by hyper proliferation of epidermis. It is a multifactorial disease with a genetic background [1]. It comprises of well circumscribed red scaly papules and plaques [2]. The disease has a major impact on the health care systems and on society in general because of its high prevalence. Due to these implications, the clinician and pathologist need to work in close collaboration to offer a diagnosis of psoriasis and to differentiate between other papulosquamous lesions. Psoriasis has many different clinical variants which mimic various dermatological conditions like secondary syphilis, seborrheic dermatitis, pityriasis rosea and parapsoriasis. The recurring nature and prognosis of psoriasis differs from other psoriasiform dermatitis. Clinical features alone are not reliable and can cause diagnostic dilemma. So, it is essential to have a histopathological confirmation of the clinical diagnosis for the satisfactory management of the conditions. Further, histological material provides evidence and can be preserved for future review. So as in other dermatological conditions, histopathology is considered as gold standard for diagnosis. [2,3,4] My study aims at evaluating the demographic profile, clinical features and clinico histopathological correlation. I also looked at the histopathological features useful in subtyping psoriasis.

Objectives

1. To assess the clinical and histopathological features of psoriasis
2. To study the clinico pathological correlation in patients with psoriasis
3. To identify the histopathological features useful in subtyping psoriasis.

METHODS

This is a case series study conducted at the Department of Dermatology OPD / IPD and Department of Pathology, Government Medical College, Thiruvananthapuram from 2011 January to 2012 December.

Inclusion Criteria

Skin biopsy specimens from all consecutive new cases received in the department with a clinical diagnosis/ differential diagnosis of psoriasis was included.

Exclusion Criteria

Those patients, who did not give consent.

Method of Data Collection

General information regarding the patient such as age, sex, age at onset, clinical presentation, duration, history of smoking and prior treatment, family history, comorbidities associated, and various histopathological parameters were studied. The skin biopsy specimens fixed in 10 % formalin received in the Department of Pathology were taken. These tissues were processed and 5 micrometer thickness sections were taken from paraffin embedded tissues. The sections were stained with haematoxylin and eosin. Detailed study of histopathologically diagnosed cases of psoriasis was done. Histopathological features helpful in identifying different types of psoriasis were assessed. Results were correlated and compared with the clinical diagnosis.

Sample Size and Sampling

All cases fulfilling the inclusion criteria who attended Dermatology Department were included in the case series. Among the 217 cases, 108 which were histopathologically diagnosed as psoriasis were studied in detail. No sampling techniques were employed.

Statistical Analysis

Data entry and analysis were done using statistical software "Epi Info". Categorical variables were expressed as proportions and quantitative variables as mean and standard deviation.

RESULTS

During the study period, out of the 217 cases in which there were a clinical/ differential diagnosis of psoriasis, 108 (49.8 %) cases were histopathologically diagnosed as psoriasis. The age ranged from 5 yrs. in the youngest to 80 yrs. in the oldest. Mean age of the patients was 44 years. The most common age group of patients in this study was between 31.25 and 55.75 years. The mean age among males was 44.2 yrs. and for that of females was 43.7 yrs. 25.9 % belong to the age category of 51-60. Majority of the patients were males (74.1 %) with a male: female ratio of 2.9:1 the mean duration of disease at histopathologic confirmation was 6.69 years. Most of the people (40.8 %) had a duration of disease between 2-9 years. Out of the 108 patients 47 (43.5 %) patients had undergone prior treatment before biopsy.

Type of Lesion	Number	Percentage
Erythematous scaly plaques	79	73
Scalp scaling	43	39.8
Nail changes	53	49
Gen. Exfoliation	18	16.6
Pustules	16	14.8
Pruritus	63	58.3
Palm & sole involvement	23	21.3
Arthritis	5	4.6

Table 1. Clinical Features

The commonest clinical features observed were Erythematous scaly plaques, pruritus and nail changes. Overall 26 (24.17) patients were smokers. But when the females were excluded (none gave history of smoking) the percentage rose to 32.5 percentage among males. Hypertension (22.9 %), diabetes

mellitus (14.8 %), both HTN and DM (7.4 %) and Coronary artery disease (6.48 %) were the commonly associated co morbidities.

Feature	Number n=108	Percentage (%)
Prominent dermal blood vessel	105	97.3
Lymphocyte as the upper dermal infiltrates	70	64.8
Normal Connective tissue & Appendages	99	91.7

Table 3. Histopathology - Dermis

Dilated and tortuous blood vessels are one of the most characteristic features of psoriasis. Prominent blood vessels were noted in 105 (97.3 %) of the cases. Most commonly (64.8 %) observed dermal infiltrates were lymphocytes. 34 (31.5 %) infiltrates were both lymphocytes and neutrophils 99 (91.7 %) participants had normal connective tissue and appendages. Elastolysis and oedema of connective tissue and appendages were noted only in 4 (3.7 %) and 5 (4.6 %) respectively.

Diagnosis	Number	Percentage
Psoriasis vulgaris	93	86.1
Pustular psoriasis	4	3.7
Psoriasis with eczematisation	4	3.7
Psoriasis with postulation	3	2.8
Guttate psoriasis	2	1.85
Follicular psoriasis	2	1.85
Total	**108**	**100**

Table 4. Different Variants Encountered in the Study

Histopathology

Parakeratosis is defined as retained nuclei in the stratum corneum layer. Parakeratosis was seen 106 (98.1 %) of cases. Confluent parakeratosis was observed in 62 (57.4 %) of cases. Acanthosis was observed in all cases. Elongated bulbous (75 %) rete ridges are a characteristic feature of psoriasis. Hyperkeratosis is defined as the thickening of the stratum, corneum. It was noted in all the cases. Neutrophil Exocytosis was observed in 66 (61.1 %) of cases. Munro Micro Abscess is defined as collection of neutrophils in the stratum corneum layer. It was seen in 41 (38 %) of the participants. Spongiosis is the presence of intraepidermal and intercellular oedema. It was noted in 83 (76.9 %) of the cases. Regular Acanthosis constituted 79 (73.1 %) of cases. Suprapapillary thinning is constituted 83 (76.9 %) of the cases. It is one of the defining features of psoriasis. Hypogranulosis is defined as the decrease in the granular layer. It was noted in 95 (87.9 %) of the cases. Kogoj abscess was observed in 6 (5.6 %) of the cases**.** It is the defined as the collection of neutrophils in the stratum spinosum layer. Bulbous rete ridges were noted in most of the cases 81 (74.1 %) of the cases. Increased mitotic figures are noted in the basal and spinous layers in psoriasis. It was noted in 80 (74.1 %) of the cases.

Histopathologic Features	Present	Absent
Hyperkeratosis	108 (100 %)	0 (0 %)
Orthokeratosis	0 (0 %)	108 (100 %)
Exocytosis of neutrophils	66 (61.1 %)	42 (38.8 %)
Munro's micro abscess	41 (38 %)	67 (62 %)
Spongiosis	83 (76.9 %)	25 (23.1 %)
Papillomatosis	6 (5.5 %)	102 (94.4 %)
Supra papillary thinning	83 (76.9 %)	25 (23.1 %)
Hypogranulosis	95 (87.9 %)	13 (12.04 %)
Kogoj abscess	6 (5.6 %)	102 (94.4 %)
Mitotic figures	80 (74.1 %)	28 (25.9 %)

Table 2. Histopathology - Epidermis

Correlation with Clinical Diagnosis

Out of the 217 cases in which a clinical or differential diagnosis of psoriasis were entertained 180 cases were histopathologically proven as psoriasis. It was seen that 64 (36 %) out of the 180 cases had a primary clinical diagnosis of psoriasis. In the rest of the cases psoriasis was included only as differential diagnosis. Few of the consistence findings in my study were hyperkeratosis, parakeratosis, regular acanthosis and dilated blood vessels. Munro's micro abscess, even though a characteristic feature of psoriasis, was observed only in 41 (38 %) of the cases.

DISCUSSION

Age

Mean age of the patients in this study was 44 years. The most common age group of patients was between 31.25 and 55.75 years. The mean age among males was 44. 2 yrs. and for that of females was 43. 7 yrs. In the study conducted by Okhandiar et al, highest incidence was noted in 20 – 39 yrs. & mean age of males & females were comparable.

Gender

There were 80 males and 28 females in our study with male to female ratio of 2.9:1. In the study by Kaur et al, Bedi et al, Nikhil Moorchung et al and Okhandiar et al were 2:1, 2.5:1, 1.2:1, 2.46:1, respectively.

Disease Duration

In our study, the disease duration ranged from 4 months -40 years with a mean duration of 6.69 years. In a study by Cemal Bilac, Aylin Turel Ermertcan et al, the duration ranged from 1 month – 40 months with a mean of 12.4 $\pm$ 9.9 months.[5]

Comorbidities

In several studies it has been found that psoriasis is associated with a number of behavioural and systemic comorbidities like obesity, hypertension, diabetes, hyperlipidemia, metabolic syndrome, cardiovascular diseases etc. As such it is important to screen for these diseases among psoriasis patients and give prompt treatment. [6] in my study, there were 16 patients with diabetes mellitus, 23 with hypertension, 9 had both diabetes mellitus and hypertension, and 7 patients gave history of cardiovascular diseases.

Clinical Features

The major clinical features of psoriasis are erythematous scaly plaques, scalp scaling, pruritus, psoriatic arthritis and nail changes which includes onycholysis and subungual hyperkeratosis. The most frequent clinical findings in the present study was erythematous scaly plaques (73 %). There were pustules in 14.8 % and generalized exfoliation in 16.6% of cases, both directing towards diagnosis of variants of psoriasis (Table. 4). Clinical Features observed by Bedi et al[7] and present study when compared Erythematous scaly plaques (90 %) present study (73 %). Scalp scaling (62 %)

present study (39.8) Nail changes (54 %) present study (49 %) Pruritus (88 %), present study (58.3 %) Palm & sole involvement (7 %), present study (21.3 %) Psoriatic arthritis (1.3 %) present study (4.6 %).

Histopathology

Histopathological features pertaining to psoriasis were studied in detail. The cardinal histopathological features of psoriasis include a combination of the following: confluent parakeratosis, regular acanthosis, scattered mitosis of basal and prickle cells, dilatation and tortuosity of dermal capillaries and mild perivascular infiltration with lymphocytes. All the characteristic feature may not be present in one section alone. [8]

Epidermal Changes

The epidermal changes described were hyperkeratosis, confluent parakeratosis and regular epidermal hyperplasia which is known as psoriasiform hyperplasia/ skin reaction pattern. Hyperkeratosis could be identified in 108 (100 %) and is one of the commonest histological features described in psoriasis. However, in a study by Puri et al hyperkeratosis was noted in only 64 % of the cases. Parakeratosis was found in 106 (98.1 %) of the cases. Confluent parakeratosis helps differentiate psoriasis from other psoriasiform reactions like pityriasis rubra pilaris. [47,48] Among these 57.4 % were confluent parakeratosis and the rest (40.7 %) were focal. In a study done by Nikhil Moorchung, JS Khullar, Manas Chatterjee, Biju Vasudevan et al, the degree of hyperkeratosis showed a strong correlation with parakeratosis.[9]

Papillomatosis, the surface elevation caused by the hyperplastic epithelium is characteristic of the variant called verrucous psoriasis.[10] it was seen only in 6 cases (5.55 %) and was mild, none of which warranted the special categorization. Regular acanthosis was seen in 79 (73.1 %) of cases. The remainder of 26.9 % of cases had irregular acanthosis. Saw toothed rete ridges forming irregular acanthosis is characteristically described in lichen simplex chronicus and lichen planus. This finding in our study could be due to eczematisation or chronic itching. [11]

Hypogranulosis, is due to the increased cell turnover, so that granular layer is thinned out in favour of the para/hyperkeratotic layers. This feature was seen in 95 (87.9 %) of cases. The rest 13 (12 %) did not show marked hypogranulosis. This may be due to treatment effect or due to resolution.

Suprapapillary thinning, another feature representing increased turnover was observed in 83 (76.9 %) of cases. Similarly, mitotic figures were seen in 80 (74.1 %) of cases. The cases with absence of suprapapillary thinning, hypogranulosis and mitosis, probably represents resolving phases of the lesion. [12,13,14]

Still other epidermal features include spongiosis with neutrophilic exocytosis and Munro's micro abscess formation. Spongiosis was seen in 83 (76.9 %) of cases. Psoriasis is primarily spongiotic and it is seen in early lesions and certain specific sites, but it never forms spongiotic vesicles as in eczematous dermatitis. The diagnosis of eczematous dermatitis is preferred over psoriasis when spongiosis is marked, but some cases have been diagnosed as psoriasis with eczematization, since the epidermal hyperplasia and type of inflammatory cell infiltrate favour the latter.[15,1]

Exocytosis of neutrophils was present in 66 cases (61.1%). Munro microabscess, could be identified in 41(38 %). In the study by Puri, Munro's micro abscess was seen in 30 % of cases. Still rarer is the collection in the spinous layer, called the pustules of Kogoj; which was seen in only 5.6 % of cases. [16,17] Puri N et al tabulated that there is a reduction in spongiosis and Munro's micro abscess in skin biopsies post treatment. Chronic plaque type psoriasis denotes stable lesions of trunk and extremities. [18] seven such cases were noted during the study. Pustular psoriasis is a rare, acute form of psoriasiform dermatosis characterized by the widespread eruption of numerous sterile pustules on an erythematous base. 4 (3.7 %) of such cases were detected which correlated with a clinical diagnosis of generalized pustular psoriasis. [19,20]

Dermal Changes

Dermis is affected earlier than epidermis in psoriasis. The earliest histopathological change is the dilatation and congestion of vessels in the papillary dermis and a mild, perivascular, lymphocytic infiltrate, with some adjacent edema. [21] In the present study dilated blood vessels were seen in around 105 (97.2 %) of cases accounting for the most consistent feature, following hyperkeratosis. Study by Puri et al showed comparable results.

Upper Dermal Infiltrates

The most common are neutrophils and lymphocytes.[22] The present study confirmed the same with lymphocytes in 64.8 % and mixed inflammatory infiltrate in the rest. Guttate psoriasis is an early lesion of psoriasis in which, the superficial dermal edema and perivascular infiltrate can be correlated with the typical clinical features.[23] two such cases were obtained during the study period. Follicular psoriasis is characterized by follicular plugging, marked parakeratosis of ostium and perifollicular and perivascular inflammatory infiltrate.[1] two such cases were identified in the present study.

Correlation with Clinical Diagnosis

Dermatology is a field with immense need for clinicopathological correlation. In the present study, cases diagnosed as psoriasis histopathologically were included. On comparison, it was seen that 38 (36%) of the 108 cases had psoriasis as the primary clinical diagnosis. In the rest of the cases, psoriasis was included only in the differential diagnosis.

Clinically florid cases show characteristic erythema and silvery white scales with Auspitz sign. Atypical or involuting or healed lesions pose diagnostic dilemma to the clinician. Although there is a need for clinical data for histological diagnosis of psoriasis, there are definite histopathological features which separate psoriasis from other papulosquamous diseases. Few of the consistent finding in the study were hyperkeratosis, parakeratosis, regular acanthosis and dilated blood vessels. [24,25] Munro's micro abscess, even though considered to be a distinct feature may be explained by the constant exfoliation of corneal layer. Some of the disparities reflected in the histopathological picture may be due to biopsies taken from quiescent lesions.

Variants

There are different clinical variants for psoriasis, including sebopsoriasis, flexural, guttate, erythrodermic and pustular psoriasis. Histopathologically psoriasis vulgaris accounted for 93 (86 %) of the cases. The variants encountered in the study were Exfoliative psoriasis, Pustular Psoriasis, Guttate psoriasis and Follicular psoriasis (Table 4). According a study by Bedi chronic plaque type psoriasis was the most common (90 %) clinical phenotype. [26]

Four cases of Generalised pustular psoriasis diagnosed histologically by the presence of spongiform pustules of Kogoj; and two cases of guttate psoriasis, by dermal edema is recorded in this study. The importance of clinical data in these cases cannot be overemphasized.

CONCLUSIONS

Psoriasiform lesions appear morphologically similar to the prototypical classical psoriasis. Depending upon the disorder, lesions vary in size, shape, scaling and configuration. However, at times, these lesions pose diagnostic dilemma to the treating clinician. The low incidence of its diagnosis and precise assessment, histopathological evaluation is essential. Moreover, in the context of treatment, it is important to differentiate between the various subtypes of psoriasis. It also has an important role in the follow up of these patients.

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Transfusion Medicine

ADVERSE DONOR REACTION PATTERN IN A TERTIARY CARE BLOOD CENTER IN SOUTH INDIA

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ABSTRACT

Aims & Objective: Primary objective is to assess the frequency and pattern of donor reaction in our blood center and secondary objective is to assess the factors associated with vaso-vagal reaction (VVR).

Materials & Methods: Retrospective observational study conducted by Blood Center of Sree Gokulam Medical College, Trivandrum. Details of the donors of the last 4 year (January 2015 to December 2019) including donor reaction details, age, gender, weight, donation status, type of donation and Blood Pressure(BP) were included in the study and were analyzed using IBM SPSS Version 21

Results & Discussion: Out of 10,647 donors, the overall donor reaction rate is 1% while in-house and camps reaction rate were 0.96% and 3.49%. Donor reaction rate in males and females were 0.99% and 1.03%. Out of 105 reactions, 70.75 % were VVR and 19 % were Hematoma. VVR rate is 0.74. Most common VVR occurred was mild type. Young age, first time donation and voluntary donation were found to be predisposing factor for VVR. VVR rate was not associated with gender, weight and Blood Pressure (BP) of the donor. VVR in post donation phase was associated with low BP and severe VVR was not associated with low BP.

Conclusion: Overall reaction rate and VVR rate were 1.0% and 0.74%. Young age, first time donation and voluntary donation were found to be predisposing factor for VVR. It is important to contact donors to report any missed delayed reaction.

KEYWORDS :

INTRODUCTION:

Donor reaction can be defined as the clinical symptoms or signs of donor discomfort severe enough to be noticed by the donor themself or the staff, respectively[1]. For whole blood donation frequency of donor reaction is 3.2 percent[2]. This event can occur before, during or after donation. Most commonly the reaction occur during the post donation phase. Injuries related to blood donation are also included in donor reactions. Vasovagal reactions (VVR) are the most common adverse reactions and the hematoma is the most common injury related to blood donation. Most of the donor reactions are self-limiting and resolves without any sequelae. Rarely these reactions require medical attention. Early and prompt management are important in avoiding donor discomfort. Occurrence of donor reaction is a demotivating factor for the future donation. van Dongen et al. study mentioned that around 9 percent of donors who experienced donor reaction did not come forward for donation again[3]. In each demography causations factors are different. First time donors, female donors, last meal more than 4 hours, inadequate sleep, inattentive phlebotomist, low weight are some of the risk factors associated with donor reaction. Better understanding of the causation factors of reaction are pivotal in prevention of occurrence of future reactions.

In our blood center, 10647 units were collected in the last 4 years (January 2016 to December 2019). 99.66 percent of the collection occurred in-house. In this study we are assessing the frequency and pattern of donor reaction and trying to assess the risk factors associated with reaction in our blood center.

AIMS & OBJECTIVES:

Primary Objective is to assess the frequency and pattern of donor reaction in our blood center. Secondary Objective is to assess the factors associated with Vaso-vagal reaction in our blood center.

MATERIALS & METHODS:

This is a Retrospective Observational study conducted by Blood Center of Sree Gokulam Medical College and Research Foundation, Trivandrum after procuring permission from Institute Research Cell and Institutional Ethics Committee. All donor reactions occurred with in the Blood Center in the last 4 year from January 2016 to December 2019 were included in the study. Donor reactions details were collected from Donor Reaction Register and Donor details and Clinical presentations were collected from Donor Questionnaire Forms. Various details of donors taken includes age, gender, weight, donation status, type of donation and Blood pressure were entered in proforma. Data cleaning was done by the principal investigation. In this study, the outcome variable is the severity of VVR in blood donors. Data entry was done in Microsoft Excel and analyzed using IBM SPSS Version 21. Univariate and bivariate analysis was done. Cross tabulation was done for bivariate analysis.

RESULTS:

Assess The Frequency And Pattern Of Donor Reaction

During the study period, there were 10,647 collections. Out of them 10,504 (98.4%) were in-house and 143 outside collections (1.6%). 9775 (91.8%) were males and 872 (8.2%) were females. Out of 10647 donors, 8662 (81.3 %) were replacement donors and 1985 (18.7%) were voluntary donors. Among total donors, 8005 (75.2%) were repeat donors and 2642 (24.8%) were first time donors. Mean age and weight of donors were 31.0 ± 9.1 and 69.34 ± 13.31 respectively. Average Systolic and Diastolic BP were 124.8 ± 13.8 and 76.5 ± 9.4 mm Hg respectively.

Adverse Reactions

Out of 10,647 donors, reactions were observed in 105 donors thereby giving an overall incidence of 1%. Out of the 105 reactions, 100 developed in-house while 5 reactions occurred in camps. Frequency of donor reaction in in-house and camps are 0.96% and 3.49% respectively. Out of 105 reactions, 96 were males and 9 were females. Frequency of donor reaction in males and females are 0.99% and 1.03% respectively. Out of 105 reactions, 76 reactions (72.4%) occurred in post donation phase, 28 (26.7%) reaction occurred during donation and 1 (0.9%) occurred before donation. Out of the 105 reactions, 75 (70.75 %) were VVR, 20 were Hematoma, 3 were Thrombophlebitis, 4 were Excessive Pain over the phlebotomy site and 2 were delayed bleeding and 1 was localized edema. Out of the 75 VVR occurred 46 were mild, 19 were moderate and 10 were severe. (See Figure 1)

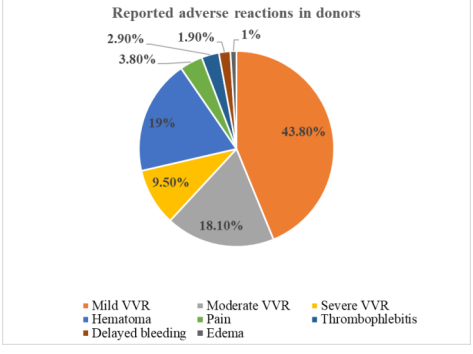

Figure 1: Percentage Of Reported Adverse Reactions In Donors

Demographic Characteristics Of Donors Who Had Reaction

The total numbers of donor reactions were 105. Mean age of the donors was 24.49 ± 6.58 years, with a range of 18–48 years. Mean age of male donors was 25.09±5.87 (range 18–42) years and female donors 29.67±11.51 (range 19–48) years. Majority of the donors were in 18-24 years age group 55.2% (58), 42.9% (45) in 25-44 years age group and only 1.9% (2) were in 45-64 years age group. The mean weight of the donors was 69.53±11.07 kg, with the weight ranging from 50 to 114 kg. The mean weight of male donors was 69.85±11.18 kg (range 50-114 kg) while, the mean weight of female donors were 66.11±9.85kg (range 50-86kg). Majority of the donors were repeat donors while only 29.5% were first time donors. Among female donors, majority were first time donors and 44.4% were repeated donors. Among male donors, 73% were repeat donors whereas gender, weight and Blood Pressure (BP) of the donors were not found to be associated with the VVR rate.

Factors Affecting VVR In Blood Donors

For further analysis, 75 donors who had VVR were selected. Around 79% of the VVR occurred after donation, 20% occurred during blood donation and only one donor had VVR before blood donation. VVR rate in donors was found to be 0.74 percent.

Age

Mean age of donors with VVR was 25.29 ± 6.35. Sixty one percent of donors in 18-24 years age category had mild VVR and 11.9% had severe VVR. In 25- 44 years category, 61.3% donors had mild VVR while 22.6% had moderate VVR. Half of the donors in more than 45 years category reported mild VVR and other half reported moderate vasovagal reaction. (See Table 1)

Gender

VVR rate in females and males were found to be 0.57 % and 0.72% respectively. Among males, sixty percent of donors had mild VVR, 25.7% moderate VVR and 14.3% reported severe VVR. Among females, 80% had mild VVR and 20% had moderate VVR. (See Table 1)

Weight

Mean weight of donors with VVR was 68.83 ± 10.5. Majority of donors are falling between weights of 60-79 kg. Nineteen percent of the donors are falling in range of 50-59 kg and 16 % are having weight 80kg or above. (See Table 1)

Table 1: Distribution Of VVR Based On Age, Gender, Time Of Reaction And Weight Of Donor

Variables	Categories	Vasovagal Reaction		
		Mild (%) n = 46	Moderate (%) n = 19	Severe (%) n = 10
Gender	Male	42 (91.3%)	18 (94.7%)	10 (100%)
	Female	4 (8.7%)	1 (5.3%)	0 (0%)
Age group	18-24 years	26 (56.5%)	11 (57.9%)	5 (50%)
	25-44 years	19 (41.3%)	7 (36.8%)	5 (50%)
	45-64 years	1 (2.2%)	1 (5.3%)	0 (0%)
Time of reaction	After donation	32 (69.6%)	18 (94.7%)	9 (90%)
	During donation	1 (2.2%)	0 (0%)	0 (0%)
	Before donation	13 (28.3%)	1 (5.3%)	1 (10%)
Weight of the donor	50-59 kg	9 (19.6%)	3 (15.8%)	2 (20%)
	60-79 kg	28 (60.9%)	14 (68.4%)	8 (80%)
	80-120 kg	9 (19.6%)	3 (15.8%)	0 (0%)

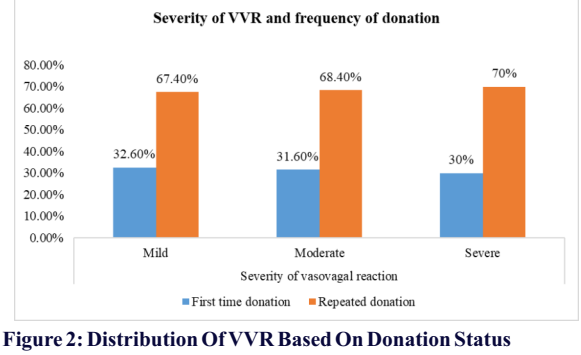

Figure 2: Distribution Of VVR Based On Donation Status

Donation Status

VVR rate in first time and repeat donors is 0.91% and 0.64 % respectively. Most of the first time donors had mild VVR, 25% had moderate and 12.5% of donors had severe VVR. Around sixty percent of the repeated donors had mild VVR, 25.5% had moderate VVR while 13.3% had severe VVR. (See Figure 2)

Type of donation

VVR rate in Voluntary and repeat donors were 0.91% and 0.66 % respectively. On examining the voluntary and replacement donations with VVR, compared to voluntary donations, replacement donations reported more donor reactions. Among donors who had severe reaction, Eighty percent were replacement donors. (See Table 2)

Table 2: Distribution Of VVR Based On Type Of Donation

Type of blood donation	Vasovagal Reaction		
	Mild (%) n =46	Moderate (%) n =19	Severe (%) n = 10
Voluntary	12 (26.1%)	4 (21.1%)	2 (20%)
Replacement	34 (73.9%)	15 (78.9%)	8 (80%)
Total	46 (100%)	19 (100%)	10 (100%)

Blood pressure

Mean Systolic and Diastolic BP of donors with VVR were 120.3± 9.73 and 77.1 ± 9.05 mm Hg respectively. Most patients who had mild, moderate and severe reactions reported systolic blood pressure less than 120. P value is reported as 0.48. Seventy five percent of the donors who had severe reaction had BP less than 120. On examining the relationship between diastolic pressure and donor reaction, seventy percent of the donors who had diastolic blood pressure more than 70 mm Hg reported severe VVR. The p value was found statistically significant. (See Table 3)

Table 3: Distribution Of BBR Based On BP

Blood Pressure	Mild VVR (%) n=46	Moderate VVR (%) n=19	Severe VVR (%) n= 10	P value
Systolic blood pressure				0.48
Less than 120 mmHg	29 (63%)	14 (73.7%)	8 (80%)	
More than 120 mmHg	17 (37%)	5 (26.3%)	2 (20%)	
Diastolic blood pressure				0.04*
Less than 70 mmHg	22(47.8%)	3 (15.8%)	3 (30%)	
More than 70 mmHg	24(52.2%)	16 (84.2%)	5 (70%)	
Total	46(100%)	19 (100%)	10 (100%)	

*p<0.05 is considered significant

DISCUSSION:

In the study average collection per annum is 2612. Majority of the collection (98.4%) eventuated in-house. In our blood center outdoor collection drive was initiated only 10 months back. Majority (91.8%) of the donors were male donors and majority (81.3 %) were replacement donors. In Indian scenario majority of donors are male[4,5].

In our study, donor reaction rate was found to be 1.0%. Various studies from India showed reaction rate varying from 0.36 to 8.3 percent.[2, 5-8] Pre-donation hydration of donors, interaction between the phlebotomist and donors during donation may be the contributing factors for this.9

Donor reaction rate for outdoor collection was higher (3.49%) compared to in-house. (0.96%). Desai et al. also reported higher reaction rate in blood donation camps.[5]Overcrowding, prolonged pre-donation phase, shortened observation time to donors, decreased phlebotomist to donor ratio, non-ambient temperature, and limited privacy to phlebotomists and donors may be the factors contributing to it.

In our study, majority of reactions (72.4%) occurred in post donation phase. Hemodynamic instabilities may be contributing factor. Most common reaction and injury transpired was VVR and Hematoma[5]. Desai et al. also reported similar results in their donor reaction study[5].

In the study, VVR rate in donors was found to be 0.74 %. Majority of the VVR were mild type. Various Indian studies showed VVR rate from 0.2 to 5 percent.[2,5-8]

According to our study younger population is prone for donor reaction as Mean age of donors with VVR was less compared to the Mean age of donors. Almutairi et al. reported similar results.[10]

VVR rate of female donors was less than that of male donors. Most of the studies concluded that females are prone for donor reaction compared to males.[10,11] In our center 350 ml is collected from female donors irrespective of the donor weight. Pre-donation hydration is strictly followed for female donors. This may be an attributing factor.
In our study, VVR rate among first time donors (0.91%) is significantly higher than repeat donors. (0.66) First time donors being young and apprehensive may be the contributing factor. Agnihotri and Almutairi et al. also concluded that first times donors are prone for VVR similar result.[10,11]

In our study, VVR rate is slightly higher in voluntary donors (0.91%) than replacement donor (0.64). Higher VVR rate in Voluntary Blood donation dives may be the contributing factor. Agnihotri et al. mentioned that VVR rate is less in Voluntary donors than replacement donors.[11]

From our study, it is shown that there was no difference in VVR rate with weight of the donor. Only 350 ml was collected from donors less than 55 kg while 450 ml was collected from donors with weight more than 55 kg. Agnihotri and Almutairi et al. reported that low weight is a pre-disposing factor for VVR.[10,11]

In our study, there was no significant difference between the mean diastolic and systolic BP of donors and donors with VVR. Majority of the VVR due to low BP occurred during post donation phase. Majority of donors with VVR had Systolic BP less than 120. Majority of donors with severe VVR had Diastolic BP more than 70mm Hg. Severe VVR may be linked to triggering factors other than BP fall. Agnihotri et al. showed that donors with low BP are prone for reaction.[11]

Some of the delayed donor reaction may be missed as there was no routine follow up of the donors. Following the study, Our center recently implemented contacting blood donors through phone the next day of donation. Pre-donation hydration, attentive trained phlebotomists, ample counselling time for first time donors and implementing donor feedback or suggestions are some of strategies implemented in our center to reduce reaction.

CONCLUSION:

Overall reaction rate and VVR rate in our blood center were 1.0% and 0.74% respectively. Young age, first time donation and voluntary donation were found to be predisposing factor for VVR. It is important to contact donors to report any missed delayed reaction.

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Rare Case of Multifocal Ileal Neuroendocrine Tumour Clinically Presenting as Mesentric Mass

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Abstract: ***Small intestinal neuroendocrine tumors (NETs) are generally slow-growing tumors and often multiple with a relatively high propensity for local and systemic metastases. We present a case of multifocal ileal NET with initial presentation as mesenteric mass.***

Keywords: NET, mesentric mass, ileum, multifocal

1. Introduction

Gastrointestinal NETs are typically low grade malignancies that arise from the diffuse neuroendocrine system scattered throughout the gut mucosa [1,2] The first description of a small bowel NET was made by Langhans, in 1867, who described a polypoid tumor of the small intestine[3]. The term "karzinoide" was first used by Oberndorfer to describe a series of six patients who had small bowel tumors [4]. The incidence of small intestinal NETs has risen steadily over the past 30 years to 1.2 cases per 1,00,000 person years clinically. Jejunoileal NETs are multifocal (2-100 tumours) in at least 1/3rd of cases[5]. We present a very rare case of multifocal NET ileum who primarily presented with a mesentric mass.

2. Case Report

A 46 year old female presented with abdominal pain of 4 months duration with no associated features of obstruction or systemic manifestations..On examination there was abdominal guarding with no organomegaly. CT scan abdomen revealed a well defined rounded homogenous lesion in mesentry in umbilical region measuring 36x33mm with fat stranding (fig1) with suspicion of mesentric carcinoid.

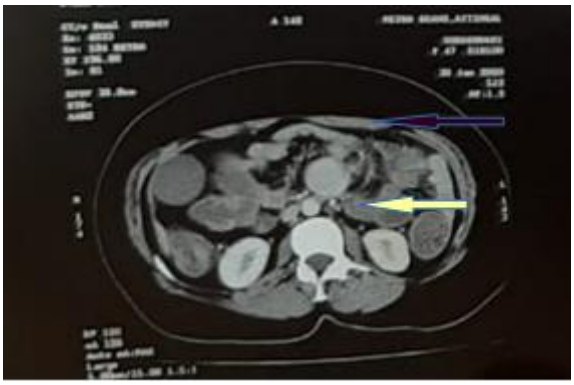

Figure 1: CT scan abdomen showing a well defined rounded homogenous lesion in mesentry

Subsequently patient underwent resection of small bowel with mesentry along with mesentricmass. Intraoperatively surgeon identifed structure involving 3cm length of ileum which was about 80 cm from ileocaecal junction (fig2).

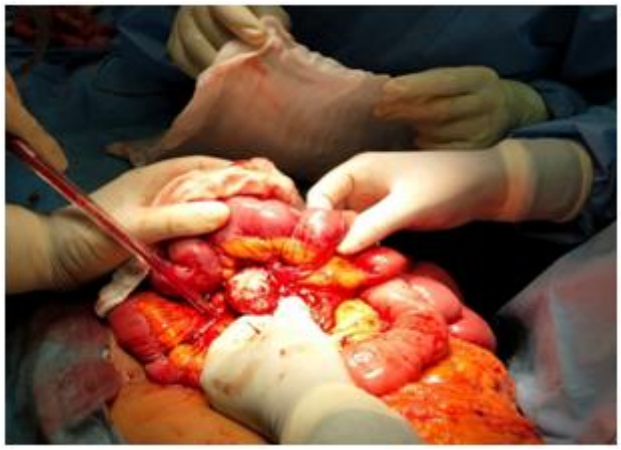

Figure 2: Mesentric mass

Gross pathological examination revealed mesentric mass measuring 4.5 x4x2.5 cm which on cut section was yellowish solid and firm. Adjacent segment of small bowel showed multiple (9) similar yellowish submucosal nodules largest measuring 2.3x1.5x1cm (fig3).

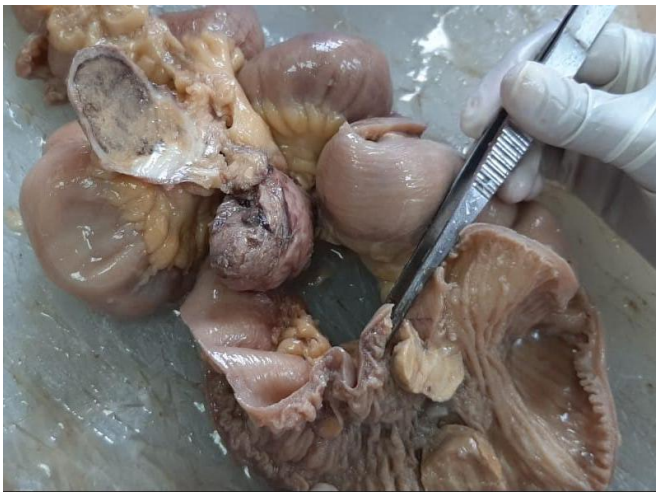

Figure 3: Small bowel with multiple NETs along with mesentric mass

The mesentry also showed 6 lymph nodes largest measuring1x1cm. Histopathology of mesentric mass and

small bowel nodules showed neoplastic cells arranged in insular and trabecular pattern with monomorphic cells having moderate eosinophilic cytoplasm and nuclei with speckled chromatin (fig4and5).

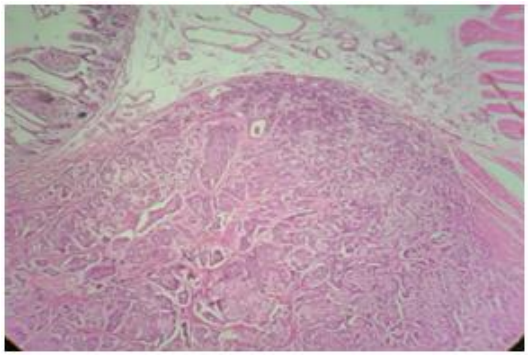

Figure 4: Neoplastic cells arranged in insular and trabecular pattern

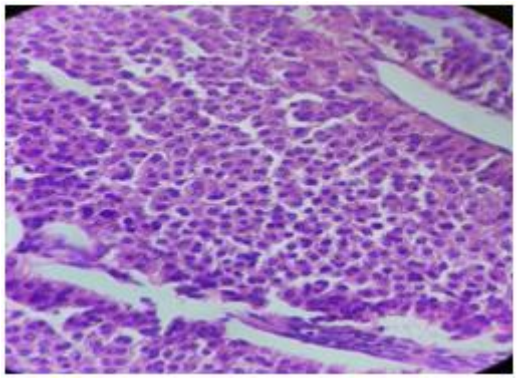

Figure 5: High power showing the speckled chromatin.

In addition the mesentric mass showed dense fibrosis around the neoplasm with infiltration into surrounding fat and a focus of perineural inavsion. The neoplasm is seen infiltrating in to the subserosa in the largest mucosal growth. The mitotic rate was 0-1 /10HPF. There was no necrosis One out of 6 mesentric lymphnodes show metastasis. So histopathological examination confirmed this case as well differentiated neuroendocrine tumour ileum grade 1, multifocal(9 in number) with infiltration in to subserosa and mesentric mass measuring 4.5 cm in greatest dimension with 1/6 lymphnodes showing metastasis..The most recent WHO classification includes NET grade 1, NET grade 2, and NEC; these are distinguished from each other on the basis of proliferative index, which is assessed by the percentage of cells that stain positive for Ki-67, and mitotic rate. Hence $T_{3\ (m)}\ N_2\ M_0$ - *Stage III* was assigned.

3. Discussion

46% to 64% of GIT carcinoid tumors arise in the midgut and most midgut carcinoid tumors originate in the terminal ileum [6].Midgut carcinoid tumors commonly spread to the mesentery, reported as occurring in 40% to 80% of cases in various series [7]..Our patient presented with abdominal pain and CT scan revealed a mesentric mass. On exploration surgeon identifed a constriction in the ileum adjascent to mesetric mass and only on gross pathology examination mutiple mucosal nodules were revealed. Tumors of the small intestine are usually discovered after resection of the bowel for symptoms of obstruction, or during exploration of the small intestine in search of a primary tumor after distant metastases have occurred[8]So when mesenric mass or lymph-node metastasis in the mesentry is suspected, we need to check the small intestine for primary lesions.[8] There was difficulty in diagnosis of mesentric mass from a completely replaced lymhnode. Since the mesentric mass has got irregular borders with invasion to fat and discontinous from the primary neoplasm a diagnosis of mesenric mass was preferred over lymph node metastasis.

MTDs were defined as discrete but irregular mesenteric tumor nodules frequently located adjacent to neurovascular bundles and discontinuous from the primary neoplasm; direct mesenteric extension from the primary tumor or extranodal extension of an involved lymph node was not considered as an MTD. Mesenteric deposits with a rounded contour or associated with a surrounding rim of lymphocytes were considered as LN metastases. MTDs are a strong predictor for liver metastasis and as a corollary, decreased disease-specific survival. In contrast, LN metastasis was not significantly associated with liver metastasis or disease specific survival.[9]

4. Conclusion

In our case the patient presented with a mesenric mass and vague abdominal pain. Only gross pathological examination revealed multiple neuroendocrine tumours of ileum.

The recently published 8th edition of TNM classification acknowledges the importance of the number of lymph nodes metastasis and the presence of mesenteric mass, incorporating for the first time this information in the N category of the TNM system. According to the new classification, presence of mesenteric neoplastic mass measuring more than 2cm in maximum diameter corresponds to N2 category, even in the absence of lymph node metastasis [10]. It is imperative to know the significance of neuroendocrine tumours presenting as a mesenric mass and its role in staging and prognosis.

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A Case of Adult Gastric Duplication Cyst Simulating a GIST on Imaging Studies

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Abstract: ***Gastric duplication cysts in adults are rare congenital anomalies and comprise about 2–9% of all gastrointestinal duplication cysts. They are often overlooked due vague symptoms. Majority of the cases are reported in children .We present a rare case of gastric duplication cyst in a 65 year old female.***

Keywords: pyloric antrum, duplication cyst, mild dysplasia, congenital

1. Introduction

Gastrointestinal duplication cysts are rare congenital disease. They are usually hollow, spherical, tubular structures, with well-developed smooth muscle coats, lined by mucosal epithelium. They develop prior to complete differentiation of gastrointestinal epithelium and as such are often named after their organ of association. [1, 2] On consideration of the fact that these cysts are usually asymptomatic or, in any case, have no specific signs and symptoms, diagnosis is frequently made post-operatively (3).Gastric duplication cysts (GDC) comprise about 2–9% of all gastrointestinal duplication cysts and most are located in the greater curvature [4] .

2. Case Report

Our case is a 65 year old female with vague abdominal pain and loss of appetite .CT scan abdomen revealed an ill defined partially exophytic lesion measuring 2.1 cm in the pyloric antrum of stomach in greater curvature possibly GIST [fig 1].

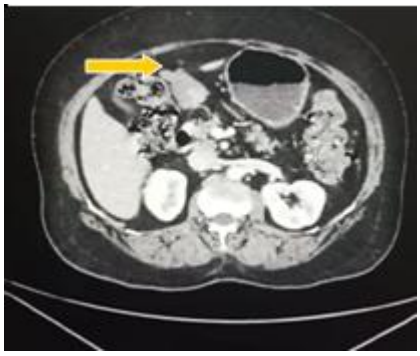

Figure 1

Subsequently the patient undergone laparoscopic wedge resection of the lesion. The gross pathologic examination showed a firm whitish nodular lesion with a central slit like space [fig2&3].The microscopy of stomach showed a lesion in the muscular layer with a central slit like space lined by gastric mucosa with one focus showing mild dysplasia, which in turn was surrounded by muscular layers. The space was not communicating with the lumen of stomach and a diagnosis of gastric duplication cyst was made [fig 4 & 5].

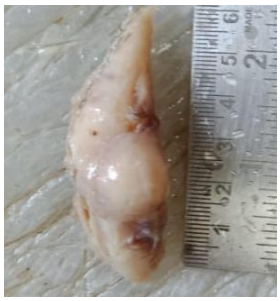

Figure 2

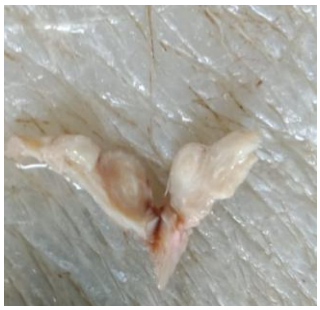

Figure 3

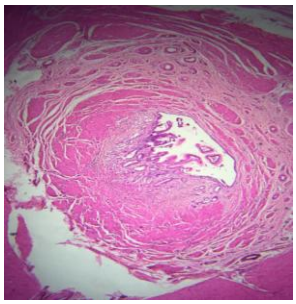

Figure 4

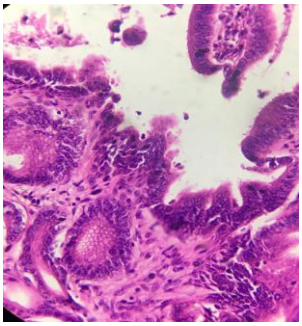

Figure 5

The patient recovered uneventfully and was relieved of symptoms.

3. Discussion

Gastrointestinal duplication cysts are rare congenital anomalies found primarily in children with majority occurring in ileum and rare in stomach. [5] Approximately 67 per cent of gastric duplication cysts are identified within the first year of life. It is very rare in adults, Kremer et al. described 9 cases, with only one adult patient [6] Duplication cysts in adults are generally asymptomatic and encountered as incidental findings at endoscopy or laparotomy [7]. Established criteria for diagnosis of gastric duplication cyst include the wall of the cyst being contiguous with the stomach wall, the presence of smooth muscle surrounding the cyst and in continuation with the gastric musculature, and lining of the cyst wall by epithelial, gastric, or gut mucosa of any type.[8]

Most gastric duplications are localised along the greater curvature. They may have a cystic or tubular configuration and may or may not communicate with the gastric lumen. Since GDC has the potential for neoplastic transformation, it is recommended that duplication cysts be surgically excised when found.[9] But there is some controversy regarding management[10]. Some authors favor conservative treatment because malignant transformation of these lesions is rare, whereas others prefer complete surgical resection even in asymptomatic patients to avoid the risk of complications such as obstruction, torsion, perforation, hemorrhage, and malignancy. [10]The diagnosis of GDC is challenging in majority of cases and diagnosis is usually made during surgical resection or by pathologic examination. On CT scan/MRI study, it is often misdiagnosed as soft tissue masses/solid tumors [10]. Our case also diagnosed as GIST by CT scan

4. Conclusion

GDC may present with vague symptoms and in radiologic examination it can easily mistaken for a soft tissue tumour of GIT So we should keep high degree of suspicion to reach an accurate diagnosis allowing appropriate treatment with total surgical resection of the lesion, when possible, avoiding future complications including malignant tumors.

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