DELTA NEUTROPHIL INDEX AND SERUM CALPROTECTIN LEVELS IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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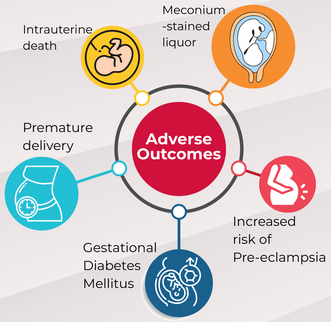
INTRODUCTION

Intrahepatic Cholestasis of Pregnancy (IHCP)

is a condition characterized by pruritus without any associated skin rash along with elevation in the serum bile acid levels ,typically in the late trimesters which resolves after delivery.

Incidence

1.3 % along with a recurrence rate of 40-70 %



DIAGNOSTIC MARKERS

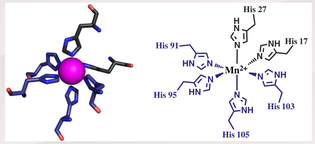
Currently, Serum Bile acid is the standard diagnostic marker for IHCP.

According to the RCOG 2022 Guidelines:

DIAGNOSIS	BILE ACID LEVELS (µmol/L)
Gestational Pruritus	<19
Mild IHCP	19-39
Moderate IHCP	40-99
Severe IHCP	>99

SERUM CALPROTECTIN

Calprotectin (S100 A8 /A9), a Ca++ Binding protein belonging to the S100 family is also known as the 27E10 antigen, protein myeloid related protein 8 of 14 related to macrophage inhibitor factor , L1 protein , & calgranulin A/B. Calprotectin is a protein that is expressed on immunocytes & stimulates the production of cytokines & leucocyte production, as seen in the case of neutrophils & macrophages.



SERUM CALPROTECTIN CALCULATION

The Serum Calprotectin were calculated using the Human Calprotectin ELISA© Cat.no E4010Hu , which was a sandwich ELISA kit for the accurate quantitative detection of human Calprotectin (also known as CAL) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates. The kit's Standard Curve Range was between 2ng/ml - 600ng/ml with a sensitivity of 1.67ng/ml.

DELTA NEUTROPHIL INDEX

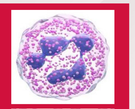
Serum delta neutrophil index (DNI), a new inflammatory marker, reflects the ratio of circulating immature granulocytes related to infection and inflammation to the total number of neutrophils.

DNI CALCULATION

·DNI (%) = (the leucocyte subfraction assayed in the MPO channel by cytochemical fraction) – (the leucocyte subfraction assayed using the nuclear lobularity channel by reflected light beam measurements) with a flow cytometry based hematolgic analyzer.

For CBC 3ml of blood was collected in the EDTA vial and the sample was processed on the same day along with a peripheral smear examination handed over to us with the heme-analyzer report . DNI values were then calculated from this report using the formula :

DNI % = (Neutrophil% + Eosinophil%) – Poly Morpho Nuclear Leucocyte %



RESEARCH STUDY

Objectives

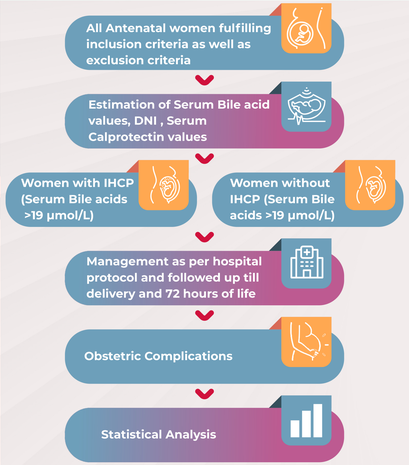
- To find the association of maternal Delta Neutrophil Index & serum Calprotectin levels with Intrahepatic Cholestasis of Pregnancy.
- To find the co-relation between both DNI & Serum Calprotectin levels with bile acids in antenatal women with Intrahepatic Cholestasis of Pregnancy.

Materials & Methods

- **STUDY DESIGN:** Prospective observational study
- **STUDY DURATION:** 18 months
- **INCLUSION CRITERIA:** Women with singleton pregnancy > 28 weeks of gestation with complaint of itching and elevated Serum liver Transaminases ALT and AST >40 IU/ml.
- **EXCLUSION CRITERIA:**
  - Dermatological pathology
  - Chronic inflammatory conditions
  - Associated comorbidities like Gestational Diabetes Mellitus, Infections, Hypertensive disorders
  - Serology positive for Hepatitis A, B, C, E
  - Patients on prior medication (ursodeoxycholic acid)

Sample Size

- The sample size for the study was based on a study by Eroglu et al who reported the sensitivity of DNI for predicting IHCP as 77.5%.
- With 95% confidence interval, the proposed sample size for the study is 86.
- Since the recruited patients who will not be diagnosed as intrahepatic cholestasis of pregnancy will serve as internal controls, a total of 172 as sample size will be taken.



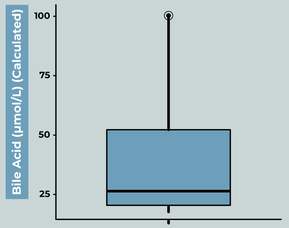
RESULTS | POPULATION STUDY

Demographics

DETAIL	CASES (n=86)	CONTROLS (n=86)
Mean Age (Years)	22.41 ± 2.86	22.34 ± 2.82
Primigravida	65 (74.4%)	66 (76.7%)
Multigravida	21 (25.56%)	20 (23.2%)
Mean Period of Gestation	38.45 ± 1.81	38.58 ± 1.55

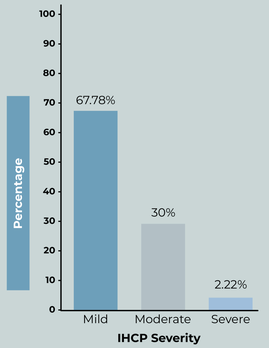
Estimation of Serum Bile Acids

BILE ACID (µmol/L)	GROUP	
	With IHCP	Without IHCP
Mean (SD)	39.50 (24.18)	10.80 (4.20)



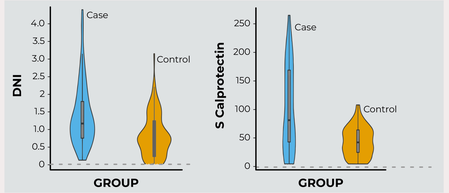
Distribution of patients with IHCP according to severity of IHCP

IHCP Severity	Frequency (n=86)	Percentage
Mild	59	67.8%
Moderate	25	30.0%
Severe	2	2.2%



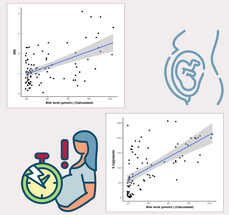
ESTIMATION OF DNI AND SERUM CALPROTECTIN IN PATIENTS WITH AND WITHOUT IHCP

BIOMARKERS	GROUP		
	With IHCP	Without IHCP	p Value
DNI Mean (SD)	1.35 (0.87)	0.78 (0.58)	<0.001
S Calprotectin Mean (SD)	98.01 (72.62)	40.27 (25.18)	<0.001



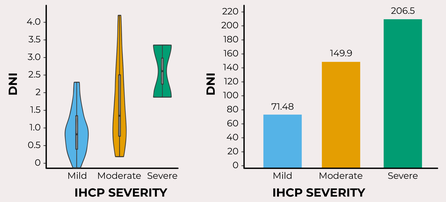
ASSOCIATION OF DNI AND SERUM CALPROTECTIN LEVELS WITH IHCP

CORRELATION WITH BILE ACID	SPEARMAN CORRELATION COEFFICIENT	P VALUE
Bile Acid (µmol/L) vs DNI	0.36 (95%CI: 0.14 to 0.55)	<0.001
Bile Acid (µmol/L) vs S Calprotectin	0.67 (95%CI: 0.52 to 0.77)	<0.001



ASSOCIATION OF DNI & SERUM CALPROTECTIN WITH GRADES OF SEVERITY (IHCP)

BIOMARKERS	IHCP SEVERITY			KRUSKAL WALLIS TEST	
	Mild	Moderate	Severe	X2	p Value
DNI Mean (SD)	1.09 (0.60)	1.83 (1.08)	2.70 (0.99)	12.904	0.002
S Calprotectin Mean (SD)	71.48 (64.01)	149.93 (56.90)	206.50 (7.78)	27.455	<0.001



DISCUSSION

EROGLU ET AL (2021) Sample Size: 40

Result : Maternal serum DNI levels were found to be significantly higher in patients with IHCP.

Limitations : Small sample size, on European population

AGAOGLU ET AL (2021) Sample Size: 40

Result : Serum calprotectin was found to be significantly higher in patients with IHCP.

Limitations : Small sample size, on European population

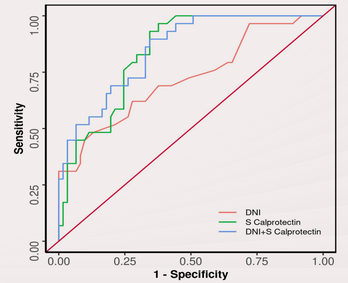
KATIYAR H ET AL (2024) Sample Size: 59

Result : Serum Calprotectin levels were higher in pregnant woman irrespective of their IHCP status.

Limitations : • Inclusion criteria took into account pregnant patients with conditions like GDM, PE.
• Stored samples of 3 years were used to evaluate the results.
• Could have lead to a natural disintegration of samples over time.
• RCOG 2022 guidelines weren't followed while labelling the patients as IHCP.

COMPARISON OF DNI AND SERUM CALPROTECTIN AS PREDICTORS OF MILD IHCP

Predictor	DNI	S Calprotectin	DNI+S Calprotectin
AUROC	0.722	0.837	0.849
95% CI	0.604-0.84	0.757-0.917	0.771-0.927
P	<0.001	<0.001	<0.001
Sn	48%	97%	90%
Sp	87%	62%	66%
PPV	64%	66%	55%
NPV	78%	97%	93%
DA	74%	73%	73%



SUMMING IT UP

STRENGTHS

- Sound statistical foundation, a study power of 90%.
- Latest guidelines followed to diagnose IHCP.
- Association of DNI & Serum Calprotectin studied for the first time in Indian population.
- Use of reliable ELISA kits & sample collection with storage at -86 degree Celsius.
- DNI is a low cost test with high diagnostic accuracy for IHCP.

LIMITATIONS

- No healthy females were taken into consideration.
- Estimation could not be done on healthy pregnant / non pregnant females.

RECOMMENDATIONS

- DNI & Serum Calprotectin can be considered as alternate global biomarkers in the prediction of IHCP.
- A standardized lab estimation technique for quantitative estimation of these biomarkers for uniformity in results.