Between tube”p”. tubes were incubated at 37°c for

Between Nov 2015 to March 2017 all consequative newly diagnosed treatmentnaïve adult active pulmonary tuberculosis patients attending district tuberculosis centre attached toAssam Medical college were enrolled in the study. Initially a total of 57 patients of of Active Pulmonary tuberculosis (APTBs) and age-sex matched 52 individuals of Latent M. tb infected (LTBIs) and 44 individuals of House hold Contacts (HCs ) wererecruited for the study. Age-sex matched LTBIs and HCs were recruited fromhouse hold contacts and individuals working in tuberculosis unit. Due to sometechncal difficulties such as weak surface staining and sample failure, analysis of Tregs and PD1/PDL1 expression study could be done on 101 individuals including 42 patients of APTBs, 26 individuals of LTBIs and 33 individuals of HCs . Patients with positive sputum smears or onepositive sputum smear and chestradiography compatible with tuberculosis and treatment naïve were considered eligiblefor study.

Liquid culture in BACTEC MGIT 320 automated TB culture system(Becton & Dickinson, BD) was done to confirm diagnosis of pulmonarytuberculosis and to do sensitivity testfor first line of anti- tubercular drugs. Patients resistant to first line ofanti-tubercular drugs were excluded from the study. Montoux and/or IGRA tests were used todifferentiate between LTBIs and healthy contacts.

All apparently healthycontacts were subjected for InterferonGamma Release Assay (IGRA) test and wasperformed according to the manufacture’s instruction using Immu Check TB Platinum version 3T (P-positive control, N-negative control and T-testing culture tube) (IMMUNOSHOP INDIA PVT. LTD). 1 mlof heparinised whole blood was added to each of the three tubes (P, N

and T)containing TB antigen in “ P” tube, background control culture tube” N” and positive control culture tube” P”. Tubes wereincubated at 37°C for 20-24 hours, centrifuged and plasma removed and stored at-20°C. The amount of interferon Gamma (IFN-?)in plasma was quantified by EnzymeLinked Immunosorbent Assay(ELISA).

Forinterpretation, the IFN- ? level in the “ N” tube is subtracted from the IFN- ? level in the “ T” tubeand Value? 14 and ? 25% of “ N” value was interpreted as positive . Montoux test was performed when requested by treatingphysician. Montoux test was performed aftercollection of blood for IGRA test.

Accordingto the standard procedures , test wasperformed with 0. 1 ml of 5TUoftuberculin PPD RT 23 and read after 72h. An induration of more than 10mm was consideredas positive test. Individuals of less than 15 years of age, apparently unhealthy and HIV positive , were excluded from the study. Institutional human Ethical clearance was obtained from the institute (noAMC/EC/PG 3519 dtd 10. 03. 2014) and after taking informed written consent, allepidemiological and clinical data were collected in patient’s informationsheets.