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A case report of false positive Bence Jones proteinuria.

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Abstract :
With only rare exceptions,Bence-Jones proteinuria reflects a malignant condition. BJP occurs frequently in patients with Multiple myeloma. It is a plasma cell dyscrasia characterised by monoclonal proliferation of B cells. When there is excess production of antibodies light chains production exceeds heavy chain production and the excess light chains get excreted resulting in positive Bence Jones proteinuria. Monoclonal production of antibodies causes a discrete, intense band in the gamma region called as M band, the hallmark for the diagnosis of multiple myeloma. Though Bence Jones proteinuria is not a prerequisite for the diagnosis of multiple myeloma, positive bence jones proteinuria points towards the diagnosis of multiple myeloma. Here we report a false positive case of bence jones proteinuria normal Electrophoresis pattern.

Keyword : Heat Precipitation Test , false positive Bence Jones proteinuria

Case report

A 60 yrs old diabetic male came with history of lower vertebral skeletal pain, fever following h/o trivial trauma. X ray showed ill defined bony lesion.He was started on antibiotics (Inj.Ampicillin,garamycin).The internist sent urine sample for Bence Jones Protein(BJP) as other blood investigations were non-contributory. Heat Precipitation Test for Urine BJP was found to be positive (Fig.1-3). Serum Protein Electrophoresis was done to confirm the diagnosis of Multiple Myeloma. M pattern was not seen in EPP(Fig.4). The curious internist again sent a fresh urine sample for BJP, which was found to be negative. Culture of the lesion confirmed the diagnosis of Fungal osteomyelitis

Discussion :

Bence Jones protein is a monoclonal globulin protein found in the blood or urine, with a molecular weight of 22-24kDa(1). It results from exclusive production of one light chain, or the unbalanced synthesis of heavy and light chains, with the latter being in excess. Free K-or -*light chains are therefore excreted alone or in addition to complete immunoglobulin molecules carrying the same light chain type. Within the kidney they are filtered through the glomeruli, reabsorbed in the proximal tubule by receptor-*

mediated endocytosis and degraded in the tubular cells by lysosomal enzymes. Light chains appear in the urine when the metabolizing capacity of the nephron is exceeded. With only rare exceptions,Bence-Jones proteinuria reflects a malignant condition. BJP occurs frequently in patients with multiple myeloma. Patients with B cell malignancies, such as Waldenstrom's macroglobulinemia, chronic lymphocytic leukemia, mu heavy chain disease, and other lymphoid tumors, not infrequently have BJP (2-4). In addition, BJP is found in association with primary systemic amyloidosis -(4-6) and light chain nephropathy (4-7). Multiple myeloma is the leading disorder, with 20% of patients excreting Bence-Jones proteinuria at presentation, and 60-80% during the course of the disease. This disease is characterized by neoplastic proliferation of plasma cells in the bone marrow and subsequently in the Multiple Myeloma has been recognized since ancient times. The first well-documented case was reported in 1844 by Samuel Solly. The most commonly recognized case is that of Thomas Alexander McBean, a highly respectable tradesman from London in 1850. Mr. McBean excreted a large amount of protein that was described by Henry Bence Jones in 1847 (8) and published in 1848 (9). Bence Jones proteins are particularly diagnostic of multiple myeloma in the context of end-organ manifestations such as renal failure, lytic (or "punched out") bone lesions, anemia, or large numbers of plasma cells in the bone marrow of patients. Bence Jones proteins are present in 2/3 of multiple myeloma cases (10). Although daily excretion of Bence-Jones protein varies widely, trends shown by monthly serial determinations furnish a useful index of progression of the underlying disease; an increment usually reflects a more aggressive form or relapse, while reduction implies responsiveness to chemotherapy. They are not detected by dipstick analysis.

If Bence-Jones protein is suspected, the heat precipitation test or immunoelectrophoresis can be performed on a urine specimen. An early morning urine sample is required for the detection as the concentration of urine is at its optimal amount .The heat precipitation test is based on the protein's unusual solubility properties. Bence-Jones protein precipitates at temperatures between 40°C and 60°C (56°C optimum), but dissolves again at 100°C. Upon cooling, the precipitate will reappear around 60°C and will dissolve again below 40°C.Free light chains usually migrate with 2 mobility on electrophoretic techniques. Identification of the isotype (K or *) requires immunoelectrophoresis or immunofixation of the urine.*

Bradshaw Test (not specific):

To add 3ml of test urine in a 5ml of concentrated hydrochloric acid containing test tube to make agood interface without mixing the two. A white curdy precipitate at the interface indicates presence of Bence Jones protein.

Heat Precipitation Test:

The test is performed by centrifuging fresh urine and then placing 10 mL in a fresh test tube. Thespecimen is adjusted to pH 5 by mixing with 25 percent acetic acid and then slowly heated in a water bath for 15 minutes. Temperature is monitored by placing a thermometer in the test tube. The formation of a precipitate at 40-60ºC indicates the presence of Bence-Jones protein. If a precipitate occurs over 60ºC, it is due to albumins and globulins. In order to separate Bence-Jones protein from albumin, the specimen is filtered at boiling temperature, thereby allowing albumin to be removed. Then the heat precipitation test for Bence-Jones protein is performed as described above.

Serum Protein Electrophoresis Procedure:

m Protein Electrophoresis Procedure:

Place two thoroughly cleaned microscopic slides on a leveled table. Weigh 100 mg of agarose dry powder and transfer to a dry test tube.

Add 10 ml of barbiturate buffer (pH= 8.6; Ionic strength= .05mmol/L) to the agarose powder and place in a boiling water bath.

Agarose powder dissolves in the buffer on boiling and the solution becomes clear.

Cool it for some time and pour 3.5 ml of agarose solution carefully onto each slide using a pipette. The agar solution finds its level in the slides and gets itself uniformly layered.

Allow 15-20 minutes for the solution to form a gel. The gel appears translucent.

Pipette a few drops of undiluted serum in a watch glass. Drop a few crystals of bromophenol blue powder over it. Mix the serum with a cover slide and stamp on to the gel at one end of the slide. Serum sticking to the coverslip is now transferred to the gel as a thin narrow line. Control serum can be applied to the other slide. Bromophenol blue is the marker dye and being a low molecular substance it will run faster than other proteins in an electrophoretic field and signify the completion of electrophoresis.

Place the slides over the bridges or the platform of the apparatus. Sample application point should be near the (-ve) end.

Pour 75-100 ml of buffer in each of the buffer trays

Cut 3.0 x 2.5 cm Whattman # 1 filter paper. Make a 1.0 cm fold. Wet the paper in the buffer and place it on each end of the gel slide. The other end of the paper wick should touch the buffer.

Connect the apparatus to the power supply. Turn the power on. Turn selector switch toward constant voltage mode. Slowly increase the voltage to 100 v.

Continue the run till the blue colour marker dye reaches the anodic end of the gel.

Decrease the voltage, stop the power and disconnect the power pack from the apparatus.

Remove the slides. Take the slides out carefully and place them in methanol for 15 mins for fixation

Remove the slides from methanol and dry it in the hot air.

Place the dried slides in a Petri dish and add the dye solution (Amido Black) to cover the entiregel. Close the tray and allow staining the gel for 20-30 minutes. During this process proteins are fixed and stained simultaneously.

After the staining is completed, that is, after 20-30 minute period drain off the stain solution. Transfer the slides to another pertridish and remove the excess stain form the slides by washing them and leaving them in destaining solution(5%Acetic acid) for nearly 20 minutes with one or two changes of destaining solution.

Take the slides out and dry them in air. Examine the bands in a white background.

False Positive Bence Jones proteinuria can be seen in patients with leukemia (blood picture,biopsy was not suggestive here), macroglobulinemia (normal EPP,no hepatosplenomegaly or hyperviscosity syndrome), H/O of high dose of penicillin (which is the case here) or aspirin or before giving the sample(11).

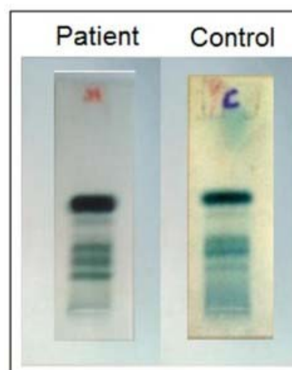
Conclusion :

This case report indicates that thorough investigations and history taking of positive Bence Jones proteinuria patients may help to rule out false positive cases . Here, we wish to alert physicians and clinical biochemists to this interference by Penicillin and stress the necessity of confirming the validity of all screening tests for Bence Jones protein by using specific electrophoretic and immunochemical tests to detect light chains.

Fig.1 Positive **Bradshaw's Test**

Fig.2 Heat precipitation test **at 55^0 c :** Appearance of Precipitation Fig.3 Resolution of the coagulum at boiling

Fig.4 Serum Protein Electrophoresis



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